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IN
EXPERIMENTAL PHARMACOLOGY

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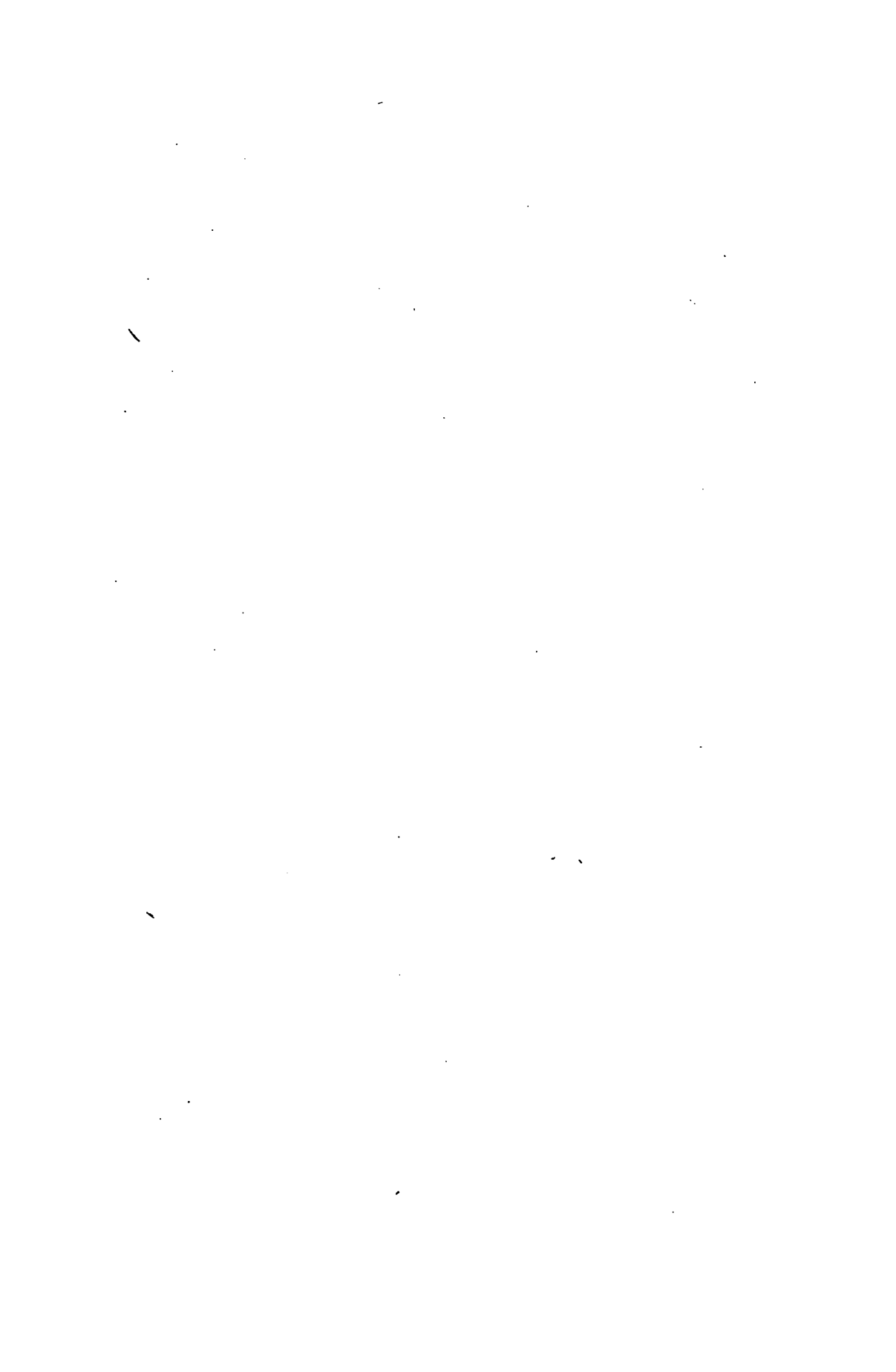
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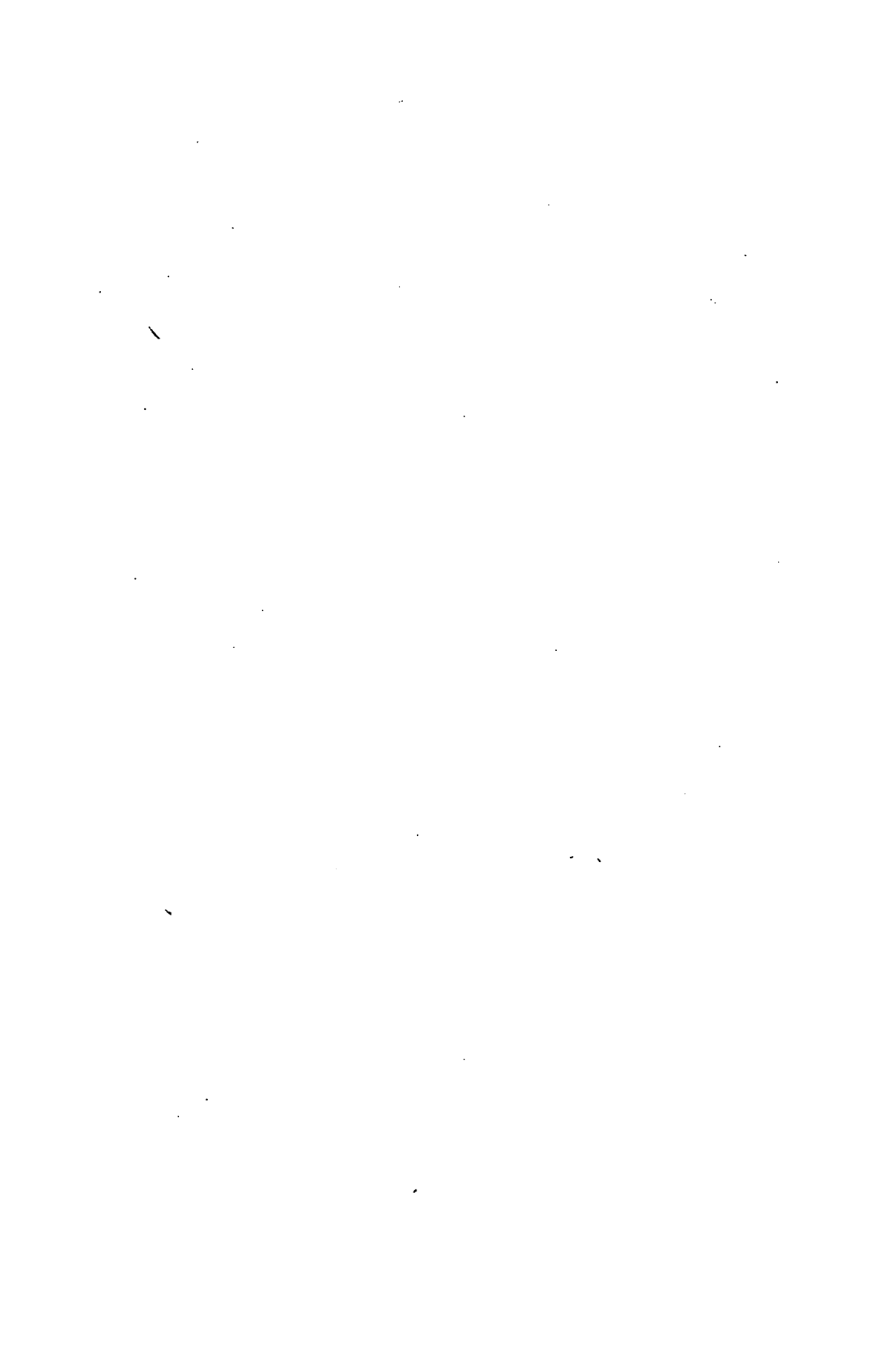
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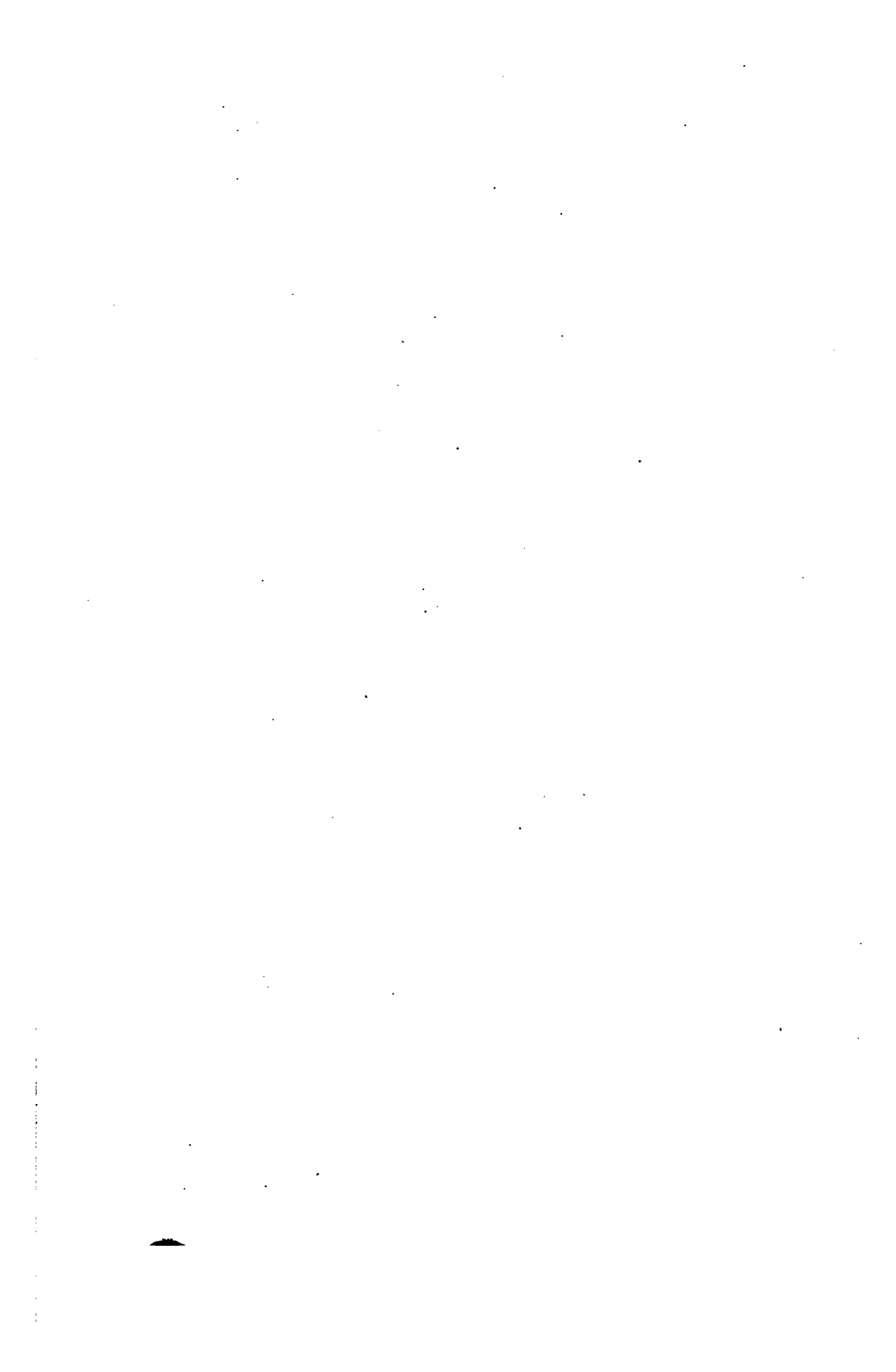
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Laboratory Guide

IN

Experimental Pharmacology

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PREFACE TO 1918 EDITION.

In the years which have elapsed since these notes were first put into permanent form the book has been placed in service in different pharmacological laboratories over the country, and in this revision which the progress of the science makes necessary, the suggestions which have been received from teachers of the subject have proved most welcome and helpful. It would seem that the wish expressed in the preface to the first edition that these notes might prove useful to others has been realized.

It need hardly be pointed out that the object of the book is not to furnish an exhaustive treatise on laboratory methods, but rather a simple outline of experiments which can easily be carried out by the average medical student in any laboratory equipped for the purpose. That the arrangement is entirely practical and workable is proved by the fact that these directions essentially as here published, have been in use in this laboratory for considerably over twenty years.

The more important changes which have been made in this revision may be mentioned shortly. The section on pharmaceutical work has been enlarged by the introduction of directions for making a few preparations other than those already included in the earlier edition of the book, and all of these directions have been collected and placed in the early part of the book. This will allow of the omission of this section entirely in schools in which medical students are given a special course in pharmacy. In case no separate course in this subject is given the students, the devotion of

a few hours to such work as is given herein will prove very instructive.

In the pharmacological work proper a section on biological assays has been introduced, and in addition directions for work on isolated tissues and organs. Attention might be called also to the experiment on the action of "Digitalis in auricular fibrillation" on account of its importance from the clinical standpoint. The number of drugs studied has not been increased as it has been felt that if the student worked out thoroughly the action of one drug as typical of a group he could work out the other members. Such an arrangement tended to simplicity and to economy in time and animals.

Finally, attention may be called to the fact that in the earlier pharmacological work the use of apparatus is reduced to a minimum, the effect of this being to train the student's powers of observation. The making of accurate protocols of the experiments in the laboratory should be emphasized as much as the making of good clinical notes in the hospital, and the former may well serve as a training for the latter.

C. W. EDMUNDS.

Ann Arbor, Mich.,
Jan. 1st, 1918.

PREFACE TO THE FIRST EDITION

The following course has been gradually developed in the pharmacological laboratory of the University of Michigan, and it has been suggested that if put in a more permanent form than hitherto it might be helpful to other teachers of this subject.

The practical course has hitherto been given as an introduction to the didactic lectures and it has been found advisable to limit the number of drugs examined to those possessing the most typical action. The object has been to train the student entering on the subject in the method of work and to impress on him that the study of drugs is to be approached in the same objective way as other branches of medicine.

The order in which the experiments are arranged is a purely arbitrary one, necessitated by the conditions of the laboratory, and can doubtless be modified with advantage to suit other classes.

A few days are devoted to purely pharmaceutical work, but the chief weight is laid on the experimental part of the course. The few hours devoted to pharmacy, however, train the student in observing the characters of the much larger number of drugs met in the lecture course, and have been found to be of considerable value. A beginning may be made in prescribing as occasion offers, but this, as well as the therapeutic applications of the results obtained in the pharmacological experiments, is better left to the teacher than expounded in the laboratory directions.

ARTHUR R. CUSHNY.

CHARLES W. EDMUNDS.

Ann Arbor, March 18th, 1905.



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The Pharmacopœia.

When a physician prescribes a certain drug from time to time in his practice it is of greatest importance to him to know that his patients are always getting the same preparation, containing the same active principles in uniform strength. These requirements could not be carried out if there were not some standard recognized by the manufacturers and followed by them in the preparation of the various medicinal remedies. Most countries possess such a standard in their "Pharmacopœias," which are generally published by the government. In this country, the government does not issue the pharmacopœia, but it recognizes its authority. The book is revised every ten years by a Committee of Revision composed of members appointed or elected by a convention of various medical and pharmaceutical societies and colleges.

It need hardly be remarked that in such a work only such remedies are included as have become well established. These are designated as "official" remedies, while drugs not mentioned are "non-official."

The pharmacopœia gives, first, the Latin title of the drug followed by the English name, and the official abbreviation, and in the case of chemicals the formula and molecular weight are given. Any synonyms are mentioned and wherever it is necessary, a short, concise definition of the drug is added, telling its nature and its source. The book then gives tests by which the identity of the drug may be recognized, and its purity and strength tested and finally mentions in what ways and in what doses it may be administered.

Official preparations are therefore made according to the directions given in the pharmacopœias and they should possess uniform strength and properties; such an ideal, however, is hard to attain, as some of the crude drugs, even when collected according to pharmacopœial directions, differ in their content of the active constituents.

General Directions.

Before giving the specific directions for the experimental work a number of general directions are given for the commoner operations which are carried out on animals some of which are usually necessary in subsequent manipulations.

The various anæsthetics employed in experimental work are also described.

Anaesthetics.

No animal is to be operated on unless it is under complete anæsthesia. Ether and chloroform may be used for this purpose, but have the disadvantage of requiring constant attention. They are therefore only used to supplement other anæsthetics which experience has shown can be used to give full anæsthesia in animals without the necessity of their being continually administered. For this purpose the following combinations of drugs usually give very good results, although in rare cases chloroform or ether may have to be given in addition.

Anæsthetic for Rabbits.

Paraldehyde, 1.7-2.00 cc. per Kg. of body weight.

While your assistant holds up the animal by all four legs and head, place a gag in the mouth and pass a stomach tube¹ through the opening in the gag, being very careful not to pass it into the lungs. Draw the paraldehyde into a pipette and place the point of the pipette in the opening of the stomach tube and blow the drug gently into the stomach,

¹ A soft rubber catheter serves very well as a stomach tube for rabbits and cats, a size of 10 or 15 F. being most satisfactory. These catheters will pass more easily if they are first moistened with water.

and then withdraw the tube. If after waiting 15 minutes the animal is not completely anæsthetized ether may be administered cautiously or a second small injection of paraldehyde given.

In place of paraldehyde urethane in doses of 1 G. per Kg. of body weight may be used as an anæsthetic. The drug is dissolved in water and given by the stomach tube.

Anæsthetic for Cats.

Chloretone, 0.3 G. - 0.4 G. per Kg. of body weight.

Dissolve the chloretone¹ in as small a volume of alcohol as possible and add about an equal volume of water. Place the animal in a cat box² and administer the chloretone solution by means of the stomach tube. The stomach tube is passed through a hole in the center of a gag which is firmly held between the jaws of the animal by an assistant.

Anæsthetic for a Dog.

Morphine sulphate, 0.1 G. to 0.25 G. Chloretone, 3 G. to 4 G.

One or two hours before the animal is needed inject the morphine solution under the skin by means of a hypodermic syringe.

¹ It is convenient to keep a stock solution of chloretone in 95% alcohol on hand so that the required amount of drug may be measured out quickly. A useful strength is the one in which one gram of chloretone is contained in each 3 cc. of the finished solution.

² A cat box is of the greatest service as a means of protection to the operator whenever this animal is used. It consists of an ordinary wooden box about 30 or 35 cm. long, 18 cm. wide and 15 or 18 cm. deep. It is provided with a sliding cover, which has a V shaped cut in the middle of one end. In the corresponding end of the box a second V cut is made. When the lid is shut these two cuts leave enough room for the animal's neck. The lid is kept closed tightly by means of a nail passed through the opposite end of the cover into the end of the box.

Just before the operation, dissolve the chloretone in alcohol and add about an equal amount of water and give it to the dog by means of a stomach tube¹ passed through a wooden gag.¹ Another very satisfactory method of giving the chloretone is to dissolve the drug in 5 or 10 cc. of olive oil which may be warmed to hasten solution. When dissolved it can be drawn up into a large veterinary all-metal syringe. While the animal is lying on its side the abdominal wall can be punctured quickly and the oily solution injected into the peritoneal cavity. Anæsthesia is produced in about 10 minutes by this method.

Operations on Frogs.

Pithing.

Before exposing the heart of a frog or carrying out any other operation upon the animal its brain is destroyed (pithed) in the following manner: Hold the frog in the left hand with its back upward, tip the head forward with the left index finger, and feel for the prominent angle made at the junction of the skull and spinal column. Just back of this angle make a cut with small scissors through the skin in the median line. Now push the sharpened end of a match forward through the foramen magnum into the skull, rotating it in the cranial cavity to destroy the brain.

Injecting into a Lymph Sac.

In the intact animal, drugs to be tested are usually injected into the anterior lymph sac. This is carried out in the following manner: Lay the animal back downwards in

¹ The average stomach tube, such as is used in clinical medicine, is about the right size for a dog. It should be about 75 cm. long.

the palm of the left hand. Hold one of its forelegs firmly between the thumb and index finger and the other foreleg between the middle and ring fingers. Draw its hind legs downward and hold them against the palmar surface of the hand by means of the little finger.

Having the drug in the glass injecting pipette,¹ which is held in the right hand, force the animal's mouth open with the point. Pass the pipette into the mouth avoiding the tongue, which is attached anteriorly and direct the point toward the floor of the mouth which with a little pressure it will pierce, entering the lymph sac. As it is pushed down the sac the point can be seen beneath the skin of the abdominal wall. The finger is now removed from the upper end of the pipette and the drug allowed to flow into the sac, or if necessary blown in.

In case the anterior lymph sac has been destroyed, for instance, when the heart has been exposed, the point of the pipette may be pushed onward and made to enter either one of the leg lymph sacs or one of the lateral sacs.

To Expose the Heart.

Pith the frog and tie it on its back on the frog board. With a pair of forceps raise the skin in the median line of the body just below the point of the sternum and make a nick in it with a pair of scissors. Starting at this point

¹ To make a glass injection pipette take a piece of glass tubing about 7 mm. in diameter and about 20 or 30 cm. long and heat the middle point in the Bunsen flame, rotating the tube constantly, and when it is red hot remove it from the flame and draw it out to a capillary size; cut it in the middle so as to leave a fine tube about 8 cm. long. Heat the large end of the pipette to smooth it off so it will not cut the tongue or lips.

cut up and outward on each side as far as the pectoral girdle and turn this V-shaped piece of skin upward toward the head. Raise the sternum by placing the forceps beneath the point and with scissors cut it through its entire length

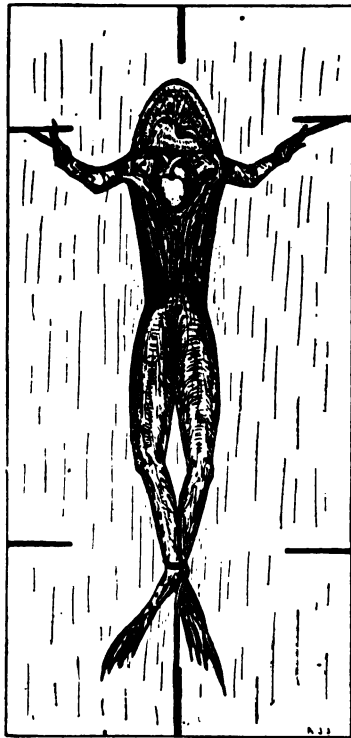


FIG. NO. 1.

avoiding injury to the underlying heart. Tighten the strings on the fore legs so as to pull the sides of the sternum well apart. The heart is now seen enclosed in the pericardium, which may be carefully opened and cut away.

Directions for Operations on Mammals.

The following are the most common operations necessary in experimental pharmacology.

To Insert a Venous Cannula.

This is to allow of the intravenous injection of drugs.

Two sizes of glass venous cannulas (with rubber tubing attached) are shown in Fig. No. 2. The larger is best suited for a dog, while the smaller is used in a rabbit or cat. Various sizes should be provided.

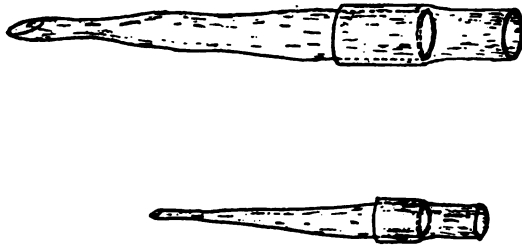


FIG. No. 2.

Operation. When the anæsthesia is complete make an incision through the skin over the site of the vessel and deepen the cut through the fascia until the vein is exposed. Avoiding injury to the vessel from forceps or tearing, clear about two inches of the vein, removing all the fascia surrounding it so as to leave it entirely free. Pass two threads about a foot long under the vein and place "bull-dog" forceps on it toward the cardiac end of the cleared portion. This allows distention of the vessel by the blood. Tie one of the threads tightly around the upper end of the cleared portion. Hold

the end of this ligature in the left hand and place the left index finger under the vessel, thus making it tense. With sharp scissors make a cut in the distended vein and place the tip of the cannula in it, and tie it in place with the loose thread previously placed around the vessel. Fill the cannula and vein with salt solution (0.8 %), taking great care that all air is expelled.

PRECAUTION. When drugs are injected intravenously it is of the utmost importance to see that no air is allowed to enter the veins. A small bubble of air is almost invariably fatal to rabbits. Be sure the cannula and syringe have all the air expelled.

To Insert an Arterial Cannula.¹

This is to allow records of arterial blood pressure to be taken.

Operation. Expose and clear the artery, usually the carotid, in the manner described above for the vein. Pass two ligatures under the vessel and tie the thread which is distal to the heart. Now clamp the artery with the "bull-dog" forceps, placing them on the "heart end" of the cleared portion. Place the index finger under the vessel as before and make a cut in it and tie a cannula in place with the loose ligature. This cannula is to be filled with sodium sulphate solution to prevent clotting of the blood.²

¹ Arterial cannulas are exactly like venous cannulas. Fig. No. 2.

² Various solutions may be used to prevent coagulation of the blood. Some of the best are as follows:

Sodium Sulphate, half saturated. This is very satisfactory and it is non-toxic, but it is rather dirty in case some is spilled.

Sodium citrate, 5-10%.

Magnesium sulphate, 25%. Very satisfactory but must be used with care as it is quite toxic in case some is allowed to enter the heart.

To Insert a Tracheal Cannula.

A tracheal cannula is inserted to allow of artificial respiration being carried on in case the chest is to be opened or if the anæsthesia is so deep that the respiratory center is seriously depressed or paralyzed.

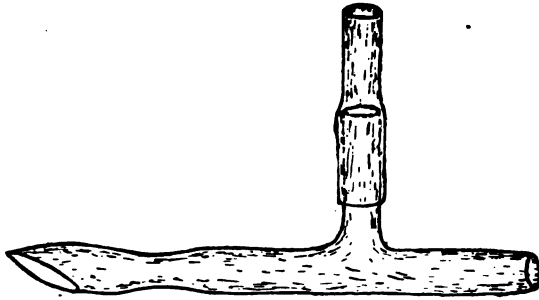


FIG. No. 3. Glass tracheal cannula with rubber tubing attached.

A convenient form of tracheal tube is shown in Fig 3. They can be made of various sizes of glass tubing so as to fit the trachea of any animal. When in use the piece of rubber tubing shown in the cut is partially constricted to give the proper degree of inflation to the lungs.

Operation. Make a median incision in the neck through the skin and fascia beginning just below the thyroid cartilage. Expose the trachea by separating the muscles with the fingers or blunt hooks and dissect away all structures from around it. Pass a strong thread under it and then make a transverse cut with the scalpel between two rings about two-thirds of the way through. Into this opening pass the tracheal tube and tie it in place with the loose ligature.

Physiological Solutions for Animal Tissues.

Physiological Salt Solution for Frogs.....	0.65%
For Mammals	0.9 %

Ringer's Solution for Frogs:

NaCl	0.6 %
KCl	0.0075
CaCl ₂ (crystals)	0.026
NaHCO ₃	0.01

Locke's Solution for Mammals:

NaCl	0.92%
KCl	0.42
CaCl ₂ (crystals)	0.024
NaHCO ₃	0.015
Dextrose	0.1

Tyrode's Solution:

NaCl	0.8%
KCl	0.02
CaCl ₂ (crystals)	0.02
NaHCO ₃	0.1
Na ₂ HPO ₄	0.005
MgCl ₂	0.01
Glucose	0.1

Chemistry of Drugs.

Alkaloids.

Among the most important of the organic compounds employed in medicine is the group of vegetable bases known as *Alkaloids*. They are found in almost all parts of plants, but in greatest abundance in the seeds and roots. They constitute in almost all cases the active constituents of the plants in which they are found and some of them are among the most powerful poisons known.

The alkaloids contain carbon, hydrogen, nitrogen, and usually oxygen, but in a few instances the latter is not present.

In their chemical properties they resemble ammonia very closely, having an alkaline reaction and uniting with acids without the elimination of hydrogen to form salts. These salts differ from the alkaloids in their solubility, and upon this fact is based one method of isolating them in a pure form from the plants in which they are found.

One of the most familiar alkaloids is quinine, which is derived from various species of cinchona bark, and it may be taken as a type of the group, as the reactions given by it are characteristic of most of the other alkaloids.

Taste quinine and quinine sulphate.

I. Test the solubility of a few crystals of quinine (alkaloid) and of quinine sulphate (alkaloidal salt) in 10 cc. of each of the following:

	Solvent.	Alkaloid solubility.	Alkaloidal salt solubility.
a	Water.		
b	Alcohol.		
c	Ether.		
d	Chloroform.		

II. Add about 100 cc. of water to 0.2 G. quinine. Shake the flask and observe only a small part goes into solution. Test the reaction of the solution with litmus. How do you explain the reaction?

Add 1 cc. dilute sulphuric acid and shake until complete solution takes place. The sulphuric acid formed the bisulphate of the alkaloid. (Note the fluorescence of the solution which is characteristic of quinine.) Use this solution in making the following tests.

III. To 5 cc. of the solution add slowly potassium carbonate solution until a distinct alkaline reaction is obtained. What happens? Render the mixture acid with dilute sulphuric acid.

Alkaloidal salts are very feeble bases and are thrown out of solution by the addition of the fixed alkalies and their carbonates which unite with the acid of the alkaloidal salt. The precipitate therefore was the freed alkaloid which is insoluble in water and which on the addition of the acid formed the sulphate (or bisulphate) again, which passed into solution.

IV. To 5 cc. of the solution add potassium hydroxide until the reaction is alkaline. Add distilled water until the test tube is nearly full and then shake the mixture and see if the precipitate dissolves.

The precipitate here, as in No. III, is the free alkaloid. The alkaloids are spoken of as being insoluble in water, but this is only relatively correct, as they are soluble in a very large excess; in the case of quinine in 1600 parts of cold water.

In the first parts of experiments III and IV it was seen that an alkaloidal salt is thrown out of solution by the fixed alkalies and their carbonates. For this reason they should not be prescribed together in the same solution, because the alkaloid would fall to the bottom of the bottle and even if it were shaken each time before a dose was taken the chances are that the last dose in the bottle would contain a much larger amount of alkaloid than was intended, and alarming if not fatal symptoms might result. The same objection holds in regard to prescribing alkaloids and tannic acids together in solution (see Exp. V a).

This incompatibility of alkaloids and alkalies may be overcome by the presence of a sufficient amount of alcohol.

V. Put 5 cc. of the solution into each of 5 test tubes and add slowly small quantities of the following reagents; note the formation of any precipitate and if any occurs test its solubility by adding water and shaking as was done in Exp. IV. Tabulate the results.

	Reagent.	Precipitate?	Soluble in water?
a ¹	Tannic acid.		
b	Picric acid.		
c	Iodine in potassium iodide.		
d	Mercury-potassium iodide.		
e	Phosphotungstic acid.		

¹ See experiment VI.

The precipitate in (a) was the tannate of the alkaloid; in (b) the picrate; (c) polyiodide (usually); (d) double iodide of the alkaloid and mercury; (e) phosphotungstate of the alkaloid. These five reagents, with a few others, of which the most important are gold chloride and platinum chloride, are known as the "alkaloidal precipitants" and they are largely used for the detection of the different members of this group.

VI. Filter the precipitate obtained with tannic acid in Exp. V a, and wash it with a little water and then taste it comparing its taste with that of quinine sulphate. Quinine tannate is sometimes known as tasteless quinine.

VII. To 10 cc. of the quinine solution add potassium hydroxide until the reaction is alkaline. Now add 5 to 10 cc. of chloroform and placing the thumb over the mouth of the test tube shake it vigorously for a minute or two or until the precipitate disappears. Set it aside for a short time until the chloroform settles in the bottom of the tube when it is to be drawn off from the water by means of a glass pipette and put into a clean, dry, evaporating dish and set aside to allow the chloroform to evaporate. Also draw off some of the water into a clean test tube and test for the presence of alkaloid by adding a few drops of the solution of tannic acid. Explain the results.

This experiment illustrates one of the methods used in separating an alkaloid from other bodies which are found present with it in the crude drug. It is known as the "Shaking out process" and will be more fully described later. (See *nux vomica* and *belladonna*.)

VIII. Boil 10 cc. of the quinine solution for a few minutes with a cubic centimeter of sulphuric acid; neutralize

the acid with potassium hydrate and apply Fehling's test for glucose.¹ Is there any reduction?

Absence of reduction shows no glucoside is present. Contrast next section (*Glucosides*).

Examine specimens of other alkaloids and their salts:—

Strychnine, from the nux vomica bean (*Strychnos nux vomica*).

Morphine, from opium, the dried juice of the unripe capsules of the poppy (*Papaver somniferum*).

Caffeine, from the berry of coffee (*Coffea Arabica*), and from tea leaves (*Thea Chinensis*).

Atropine, from deadly nightshade (*Atropa Belladonna*).

Berberine,¹ from golden seal (*Hydrastis Canadensis*).

Coniine,² from the fruit of the poison hemlock (*Conium maculatum*).

Nicotine,² from tobacco (*Nicotiana tabacum*).

Notice that the names of alkaloids end in "ine," in Latin "ina," as for example, strychnina, morphina, etc.

Glucosides.

Next in importance to the alkaloids among the vegetable poisons rank the *Glucosides*. These are substances which break up when acted on by dilute acids or ferments, and yield as one of their decomposition products a sugar which in many cases is glucose; in fact, glucosides may be regarded

¹ Fehling's test for glucose is carried out as follows: Mix in a test tube equal quantities (about 1 cc.) of two solutions, one, a solution of copper sulphate, and the other, a solution of potassium hydroxide and Rochelle salts. The resulting mixture is a clear, dark blue solution, which is to be boiled and the solution to be tested added to it. If glucose is present a red precipitate of cuprous oxide will be thrown down after the solution has stood for a short time. The precipitate may be clearly seen in the bottom of the test tube if a reducing substance is present.

¹ Berberine is one of the few colored alkaloids.

² Coniine and nicotine are the two most important liquid alkaloids. They contain no oxygen and are volatile.

as salts of sugar. The other products differ in the different glucosides, in some cases being a volatile oil, in others an alkaloid, etc.

In the following experiments amygdalin will be employed. It is a glucoside which is found in bitter almonds, laurel leaves and in the bark of the Virginian prune or cherry.

Make 50 cc. of a 2% solution of amygdalin in water.

I. Apply Fehling's test for glucose (see page 28, footnote) to 5 cc. of the amygdalin solution. Is a reducing substance present?

II. Boil 10 cc. of the amygdalin solution with 1 cc. of sulphuric acid (dilute) for 10 minutes. Neutralize with sodium hydrate and apply Fehling's test. Do you get any reduction? Compare with Exp. I, and explain.

III. To 5 cc. of the amygdalin solution add some saliva and place it on the water bath at 40° for half an hour. Test for a reducing substance as in 1 and 2 and explain results.

IV. Put about 5 cc. of the amygdalin solution into each of 5 test tubes and test each with the alkaloidal precipitants.

	Reagent.	Precipitate?
a	Tannic acid.	
b	Picric acid.	
c	Iodine in potassium iodide.	
d	Mercury-potassium iodide.	
e	Phosphotungstic acid.	

V. Pulverize three bitter almonds in a dry mortar and place the powder in a small beaker and add about 10 cc. of warm, not hot, water to it. Boil three more bitter almonds for two or three minutes in about 10 cc. of water and then pulverize them in a mortar and add warm water as in the

first instance. Set both mixtures aside for a short time and compare the odors from the two beakers.

The odor found is due to the oil of bitter almonds formed by the decomposition of the amygdalin through the action of a ferment, emulsin, also present in the almonds and acting in the presence of water. This oil consists of benzaldehyde and prussic acid and is therefore extremely poisonous.

What reaction is characteristic of the glucosides?

Examine specimens of other glucosides:—

Salicin, found in the willow and poplar.

Digitalin, found in the fox glove (*Digitalis purpurea*).

Digitoxin, found in the fox glove (*Digitalis purpurea*).

The names of glucosides end in "in," in Latin "inum," as for example, salicinum.

Volatile Oils.

The group of volatile oils contains a large number of preparations which are extensively employed in medicine. They are also known as the *Essential or Ethereal oils*. They are found widely distributed in nature as the odor of the various plants and flowers is due to them or to their oxidized products. In their chemistry they differ greatly from the group of fatty or fixed oils from which they must be carefully distinguished, the latter being non-volatile. The volatile oils are not to be regarded as pure substances, like the alkaloids, but rather as mixtures of varying composition. Most of them are colorless when fresh and pure, but they tend to become colored after long exposure to light and air. In odor and taste they differ widely from one another, but in both cases these properties are generally characteristic of the individual oil. The majority of them are lighter than water, there being only a few heavier.

As a typical member of the group the oil of turpentine is to be employed in the following experiments. As a type of the fatty or fixed oils cotton seed oil will be used to illustrate some of the differences between the two series.

I. Solubility of volatile oils.

Into each of five test tubes put 1 cc. of the oil of turpentine and then add 5 cc. of the following reagents. Shake and note whether the oil is soluble.

	Reagent.	Solubility?
a	Water.	
b	Alcohol.	
c	Ether.	
d	Chloroform.	
e	Cotton seed oil.	

II. Solubility of fixed oils.

Into each of 5 test tubes place 1 cc. of cotton seed oil and then add 5 cc. of the following reagents. Shake and note the degree of solubility as in I.

	Reagent.	Solubility?
a	Water.	
b	Alcohol.	
c	Ether.	
d	Chloroform.	
e	Oil of turpentine.	

What differences in solubility exist between the two series?

The two groups of oils resemble one another in leaving a greasy stain when dropped on paper, but that from the fixed oil is permanent.

III. a. Put a drop of oil of turpentine on glazed paper. Note the stain. Hold the paper from six inches to a foot above the flame of the Bunsen burner for a few minutes. Result?

b. Repeat, using cotton seed oil, being careful not to use enough heat to char the paper. How does it differ from the previous experiment?

IV. Decomposition product.

a. Heat 5 drops of the oil of turpentine in a dry test tube. Note the odor.

b. Heat strongly 5 drops of cotton seed oil in a dry test tube. Note the odor.

In the latter case acrolein is formed from the partial decomposition of the fat and is recognized by its irritating and disagreeable odor resembling burnt fat.

V. Add 1 cc. oil of turpentine to 5 cc. water and shake the mixture well for a minute or two. Allow the oil and water to separate, and then draw off the latter with a pipette into a clean test tube. Note its odor and taste, which are due to the turpentine, of which the water has taken up a small amount, forming a preparation recognized in the Pharmacopœia as an "*Aqua*." These waters flavored by the various volatile oils are largely used to give a pleasant taste and smell to medicines.

The "*Syrupi*" are solutions of sugar and water to which is often added a small quantity of a volatile oil as in the aquæ.

Spiritus, spirits, are solutions of a volatile oil in alcohol (Exp. I b.); they are largely used in medicine as flavoring agents.

Examine specimens of the different volatile oils named below and compare them with the oil of turpentine as regards odor, taste, color, etc.

Oleum Menthæ Piperitæ (oil of peppermint).

“ Gaultheriæ (wintergreen oil).

“ Lavandulæ Florum (oil of lavender).

“ Eucalypti (eucalyptus oil).

“ Caryophylli (oil of cloves).

Resins.

Among the less important chemical compounds used in medicine are the *Resins*. This is a miscellaneous class characterized by the smooth, shining fracture.

Using powdered resin test its solubility as follows:—

	Reagent.	Solubility?
a	Water.	
b	Alcohol.	
c	Ether.	
d	Chloroform.	
e	Oil of turpentine.	

Add water to b and explain result.

Examine the Resin of jalap.

Resin of copaiba.

Oleoresins are solutions of resins in volatile oils.

Examine Turpentine.

Burgundy pitch.

Copaiba.

Balsams are like oleoresins, but contain also benzoic and cinnamic acids.

Examine Balsam of Peru.

Balsam of Tolu.

Gum resins are mixtures of gums and resins, containing usually in addition some volatile oil.

Examine *Asafoetida*.

Gums are amorphous transparent substances belonging to the group of carbohydrates. Some dissolve in water while others only swell to a jelly. They are insoluble in alcohol.

Examine *Acacia*.

Tragacanth.

Saponins.

In a large number of plants are found certain substances of a glucosidal nature which because of close chemical and pharmacological relationships have been included under one group known as Saponins or in the case of the more toxic ones, Sapotoxins. Chemically many of them may be arranged in a series $C_nH_{2n-8}O_{10}$ and they all possess the characteristic glucosidal reaction, being decomposed by acids and ferments with the formation of a reducing substance.

They possess the property of forming a soap-like solution with a very persistent foam when even very dilute watery solutions are shaken. It is on account of this property that the plants from which some of them are derived have obtained their popular names such as Soapwort (*Saponaria officinalis*); Soapbark (*Quillaja Saponaria*), etc.

They also aid in the emulsification of fats and in the suspension of fine powders and act powerfully in dissolving red blood cells.

Using a 2% solution of saponin make the following tests:—

I. To 1 cc. in a test tube add 5 or 10 cc. of water. Shake well and then set aside noting the persistence of the foam.

II. To 2 cc. castor oil add 1 cc. of the saponin solution and shake well. Now add about an equal volume of water and shake thoroughly. A uniform white mixture consisting of the free fat droplets held in suspension by the saponin results. Set the emulsion aside for a short time and see if the oil separates. Later add to the emulsion about half as much alcohol and after shaking set the mixture aside and see if any change results.

Percolation.

The U. S. Pharmacopœia gives the following definition of this process: "The process of percolation consists in subjecting a comminuted substance or mixture of substances, contained in a vessel called a percolator, to the solvent action of successive portions of a liquid termed the menstruum in such a manner that the liquid, as it traverses the powder in its descent to the receiver, shall extract the soluble constituents and pass from the percolator free from insoluble matter."

The liquid with the soluble portions of the drug dissolved in it is known as the percolate.

The drug to be percolated is usually ground to a fine powder in order to break up the vegetable cells, etc., so that the menstruum can penetrate it easily, and the menstruum is allowed to pass through the drug only very slowly, giving it time to dissolve the soluble constituents. To still further aid this penetration, a common plan is to moisten the powder with a small quantity of the menstruum and to allow it to remain tightly covered (macerate) for twenty-four or forty-eight hours before beginning the percolation. This also gives time for the drug to swell, as is likely to occur in some cases.

Percolators¹ are usually cylindrical, or conical, and vary in size with the amount of drug to be exhausted. The lower orifice is to be closed with a tightly fitting cork, which is best put in from the inside, but this is not necessary

¹For all purposes in this course the cylindrical percolator of the following dimensions is large enough: Length, 28.2 cm.; internal diameter at top, 3.7 cm.; capacity, 275 cc. It should be provided with an ordinary iron stand and ring, the latter being padded with cotton.

with the small percolators. Through this cork passes a small glass tube (5 mm. in diameter), the inner end of the tube being flush with the top of the cork. To the outer end of the tube is fitted a piece of rubber tubing. One method of regulating the flow of the percolate is to have this tubing of sufficient length to reach nearly to the top of the percolator and to have the free end fitted with another glass tube; the latter can be raised or lowered until the percolate escapes at the desired rate. The other method, which probably is not quite as good, is to have the rubber tube about an inch long and to regulate the flow by means of a small screw clamp.

Having fitted the cork and tubing the percolator is ready to pack if the drug has macerated the required length of time. The packing is done by means of a plunger, which is made by taking a cork with a diameter about half as great as the diameter of the upper opening of the percolator and sticking into it a glass rod which is a little longer than the depth of the percolator.

Place in the bottom of the percolator a small piece of absorbent cotton and press it loosely in place. Only a small amount of cotton should be employed, as its only use is to keep the powdered drug from passing down and clogging up the exit tube. Now put in the drug, packing it uniformly in place with the plunger. Do not use much force or the drug will be so tightly packed that it will be very hard for the menstruum to pass through. Very little more force will be required than would be given by dropping the plunger from a height of a few inches. Experience together with a knowledge of the physical and chemical constitution of the different drugs can alone teach how much force to employ. When the drug is in place cover it with a piece of filter paper cut

so as to roughly fit its upper surface and weight it down with a small glass stopper. Pour in the menstruum to the top of the percolator and cover the latter with a small glass plate. If the lower orifice is open the menstruum will soon penetrate through the drug and begin to drop from the rubber tube. In cases in which the formula prescribes that the drug is to macerate for a certain length of time in the percolator, the clamp is closed, or if a long tube has been used the end is raised to a level which will prevent any outflow. At the end of the prescribed time allow the percolate to escape, receiving it in a bottle or flask. Regulate the outflow so that it shall not escape faster than ten or fifteen drops in a minute. It is best after once starting percolating to keep a layer of menstruum above the surface of the drug so as to prevent the access of air to its interstices. If the latter happens the drug contracts into irregular masses leaving furrows and fissures, and when more menstruum is added it will naturally flow through these channels and will not penetrate the drug evenly.

The U. S. P. gives general directions for percolation and also in the cases of the individual drugs specifies the size of the powder and the number of grams to be used together with the amount and character of the menstruum. It also gives directions as to the length of time the drug is to macerate before percolation.

Percolation is one of the most important processes employed in pharmacy, as by it are prepared extracts (page 147); fluid extracts (page 96); and tinctures (page 168), and it will be used in the manufacture of these preparations in the following pages. In addition it is one of the steps employed in the isolation of alkaloids.

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Powders.

A very considerable group of medicinal substances is prescribed in powder form. Some substances act better when given in this manner and certain individuals also find them easier to swallow than either pills or capsules. To be best suited for this form of medication the drug should be fairly insoluble so as to avoid taste and should not be given in too large a dose.

The manner in which powders are dispensed may be learned best by filling a prescription such as the following in which 100 mg. of calomel are to be divided into eight powders. On account of the weight of the calomel the bulk of each powder has to be increased by another powder which is inactive; in this case, sugar of milk being used.

R

Hydrargyri chloridi mitis 0.1

Sacchari lactis 1.5

Misce et divide in pulveres No. VIII.

Sig.

Take one powder every 20 minutes until all are taken.

Weigh out both ingredients and put the calomel into a small mortar and add the sugar of milk, little by little, mixing it all the time with the pestle, using only light pressure to avoid the formation of compact layers. From time to time, scrape the powder together into the middle of the mortar. When the two ingredients are thoroughly mixed, empty the powder onto the pill tile and without compressing it at all arrange it in an oblong parallelogram, the longer edge being near the scale on the tile. See that the powder

is of a uniform depth over the whole mass and if it is not, scrape some powder very lightly from the high points down to the thinner parts. The powder can now be divided easily into eight equal portions by using the spatula with reference to the scale on the tile. The powders are now ready to be put in papers which are obtained by the dispenser all ready for use.

Take eight papers and make a narrow fold in each along one of the longer sides. Put a powder in the center of one of the papers and bring the uncreased edge of the paper over into the fold previously made and then both of these are brought over to the center, creasing the whole paper along the lines formed. The ends of the paper are now creased by pressing them over the sides of a powder box and one end is tucked within the fold of the other. Fix all the powders in the same way and place all in the box, labeling it properly.

Capsules.

Capsules are shells of gelatine into which medicinal substances are put in order to cover up the taste. The substances placed in the capsules may be either in solid or liquid form. Empty capsules made of gelatine are purchased by the pharmacist so that the problem is merely one of filling. The process can be best learned by the experience gained in filling one or two prescriptions.

R

Quininæ Sulphatis 1.0

Pone in capsulas No. VIII.

Sig.

Take one capsule every three hours.

To fill this prescription the quinine sulphate is weighed out and placed upon the pill tile and spread out into an oblong parallelogram with the powder of uniform depth. This mass of powder is now divided lengthwise with the spatula and then crosswise in the middle and then on each side of the middle, thus making eight equal piles of quinine. The large end or bottom part of the capsule (size No. 2) is now pressed down on the powder repeatedly until all the powder in one of the piles is packed into it and then the top part is placed on the base, thus closing it. The process is repeated with the other seven capsules until all are filled. All traces of quinine must be removed from the outside of the capsules either by wiping carefully with a clean soft cloth or washing them in alcohol and then drying with the cloth.

There are, of course, various modifications of this method of filling capsules, made necessary by the different char-

acteristics of the individual powders which it may be desired to place in the capsule. For example, some powders lack adhesiveness and would run out of the shell each time it was raised. Such powders may be put in by means of the spatula or by the use of some form of filling apparatus -- many of which are on the market.

Oily or alcoholic liquids may also be dispensed in hard capsules as for example in the following prescription:

R

Olei Terebinthinæ 4.

Pone in capsulas No. VIII.

For this purpose select eight empty capsules (size No. 0) and remove the tops, placing them mouth downwards upon some moist filter paper. This will soften the gelatine at the point of contact with the moisture. Draw up some of the oil of turpentine into a one or two cubic centimeter graduated pipette and taking the bottom part of the capsule between the thumb and first finger of the left hand, allow 0.5 cc. of the oil to flow into the capsule, taking care none gets on the outside. Now replace the top, thus closing the capsule, and rotate it between the thumb and finger to seal it by compressing the soften ring of gelatine which was at the mouth of the top portion. Repeat the process with the other capsules.

Soft or elastic gelatine capsules are also employed in the dispensing of oily liquids.

Cachets.

A convenient mode of prescribing powders, especially those which have a disagreeable taste and are not to be given in too large doses, is by enclosing them in cachets which are small concave discs made of flour. The powder is put between two of the discs and the edges then sealed forming a tight compartment.

Fill the following prescription:—

R

Quininæ Sulphatis 0.5

Pone in cachetas No. III.

Sig.

One cachet every three hours.

Weigh out the quinine sulphate and placing it on a pill tile divide it into three equal portions. Holding one of the paste discs in the left hand, by means of the spatula place one of the powders of quinine in the middle of the disc, taking care none of the quinine gets on the edge of the disc. Moisten the edge of another disc with a very little water, and then place it against the edge of the disc which holds the quinine. Press the two edges together firmly, thus sealing the cachet. Fill the other cachets in the same manner and remove any quinine which may be on the outside. These cachets may be swallowed very easily if they are first softened by being placed in water for a few moments.

Pills.

Pills are spherical or ovoid masses of medicinal substances of a size convenient for swallowing. In weight they should not exceed 0.3 G., the average being about 0.2 G.

This method of administering solid medicinal substances is perhaps more common than any other, partly on account of convenience and also because substances which have a disagreeable taste may be given most agreeably in this way. Drugs which are corrosive or are very deliquescent or which have to be given in very large doses should not be given in pills. Those which are best suited for administration in this form are powders and extracts.

The first step in pill making is to form the *pill mass*, which should be of doughy consistency but not sticky. The mass consists of two parts; the active ingredients which are to be incorporated in the pill and some substance (the excipient) which will give the proper degree of consistency and adhesiveness. The excipient will vary with the character of the active ingredients. Some of the powdered extracts only require water to give them the proper consistency, while dilute alcohol may be needed in other extracts. Some fluid preparations may be made into pills by using an inert powder and on the other hand a non-adhesive powder may be made into a pill mass by the use of soft extracts or by the use of such an excipient as a combination of glycerin and tragacanth (glycerite of tragacanth). The choice of an excipient is very important, as it largely determines the usefulness of pills if they are kept for some time. For example, a pill made with acacia as excipient

may become so hard that it will pass through the body unchanged, while with other excipients the pill may dry out and crumble to pieces after they have been made for a short time. The formation of a pill mass may be learned best by filling the following prescription, which calls for ten pills to be made from a gram of extract of gentian.

R Extracti Gentianæ, 1.

Divide in pilulas, No. X.

Counterpoise two slips of paper on the scale pans and weigh out the correct amount of extract, which is then transferred to a mortar. (If some of the extract sticks to the piece of paper moisten the *opposite* side of the paper with water and wait a few moments, after which the remains of the extract may be easily scraped off.)

Make the extract into a pill mass of the character described above. If it is too hard add a little water or dilute alcohol; if too soft add a small quantity of a powder, such as starch or the powdered extract of liquorice. Triturate the mass thoroughly in the mortar (not in the hands!) so as to get the two substances uniformly distributed through each other. When the mass is completed, work it up with the hands into a short cylinder and transfer it to the pill slab, over which has been scattered a small quantity of a dusting powder, such as lycopodium. Roll out the mass with the wooden roller into an even cylinder of the right length to correspond with the scale on the slab (in this case roll out to 10), taking care that the ends of the cylindrical mass are "squared up." With the cylinder over the scale, cut it with the spatula into the required number of pieces, taking care that the spatula is always held at right angles to

the mass. The little sections of the pill mass are now to be moulded with the thumb and fingers into spherical shape and then placed in the pill box with a small amount of lycopodium and shaken about so that they may be completely covered by powder which will prevent them sticking together. Discard the excess of powder.

To mask the taste of the pills they are usually coated with some substance such as gelatin, sugar, chocolate, etc.

Examine the pills after a day or two and see if they have retained their shape or if they have flattened out. Weigh five pills and see how nearly they correspond in weight.

Emulsions.

Emulsions are aqueous preparations in which oils, resins or insoluble powders are suspended by means of gummy substances (emulsifiers).

The object of emulsification is to render substances insoluble in water freely miscible in that fluid, so that any disagreeable taste may be more easily disguised and the absorption of the oil rendered more easy. In nature, some emulsions exist already formed, as for example, milk, the juice of plants, the yolk of eggs, etc.

There are a large number of substances which can be used as emulsifiers, the most important being acacia. Condensed milk and yolk of egg are superior to acacia for this purpose, but possess the disadvantage of becoming stale.

In some substances, as for instance in gum resins, the gum needed to form an emulsion is found present with the resin. In the seeds of some plants albuminous substances are found and these act as emulsifiers of the oils present in the seed, rendering the addition of gums unnecessary.

The various types of emulsions are prepared according to the following directions:

I. Seed emulsion prepared from bitter almonds.

Take four or five bitter almonds and remove their skins. This may be done in two ways. They may be dropped for a few moments in very hot water and then in cold, when the skins can be slipped off. The danger with this method is that the boiling water may destroy the ferment, emulsin, which aids in the emulsification. To avoid this, the seeds

may be soaked in lukewarm water for half an hour, or until their skins are loosened.

Break up the seeds and put them in a clean mortar and add a small quantity of water (one part to ten of seeds) and with the pestle rub the seeds up into a thick, creamy paste which shall be free from lumps. More water can be added now, gradually, until a milky solution is formed, which may be strained if necessary.

II. Emulsion of a gum resin.

Prepare 100 cc. of the emulsion of asafœtida according to the U. S. P.

III. Emulsion of a fixed oil.

Among the various methods employed in making emulsions, that known as the Continental is probably the most satisfactory, and is to be used in the following experiment.

Prepare 60 cc. of a 50% emulsion of castor oil.

In this method a primary emulsion or nucleus is first formed with certain proportions of the ingredients and this nucleus can then be diluted with water without the fear that separation will occur.

The proportion by weight is as follows:

Oil	4
Water	2
Gum (powdered acacia)	1

Using this formula, calculate the amount of each ingredient needed and weigh them out carefully in clean vessels. Pour the oil into a clean, dry mortar and add the acacia, and with the pestle stir the two together rapidly until a uniform mixture is obtained. (This must be done quickly, as other-

wise the oil and acacia remain in contact too long and the latter tends to become hard and insoluble. The object is merely to get each particle of gum incased in oil.) To the oil-acacia mixture add *all* the water (as determined by the above formula) and triturate the mixture rapidly until it becomes a thick, white, creamy emulsion. In the trituration, which should take from 3 to 5 minutes, no pressure is needed, but a rapid motion is very important. The pestle should be held loosely between the thumb and first two or three fingers and the motion given not only from the shoulder and elbow, but also from the wrist and fingers. It is best to move the pestle always in one direction, but it is not essential to do so.

When the nucleus is complete it can be poured into a bottle and water added to make up the required bulk. It should then be well shaken.

Such an emulsion may separate after some hours into two layers without showing any separated oil. Shaking will restore the uniform appearance. Such separation is entirely analogous to that which occurs in milk when the cream rises to the surface.

Emulsions can be flavored by various agencies, such as the volatile oils, but, as a general rule, some of the less powerful flavors will disguise the taste of the oil better, as for instance, liquorice extract, coffee, vanilla, or chocolate. They may be sweetened by small quantities of sugar or saccharin.

Acids, alcohol, or glycerin, if added in any except the smallest quantities, cause the emulsion to separate (break).

Ointments.

Ointments are soft fatty preparations which are intended for application to the skin. The purpose of an ointment may be merely to act as a protective to the skin or to render it softer and more elastic, in either case the action being merely a local one. Then, too, active substances are sometimes incorporated into ointments in order that they may be absorbed and exert a general or systemic action upon the body.

In order that ointments may be used for these different purposes, it is evident that substances possessing different properties must be used in their formation. In one instance it is not desirable for the ointment to be absorbed, whereas in another case lack of penetrating ability might be disadvantageous. To give these different properties to ointments, there are three substances used most commonly in their formation; namely, vaseline, lard (or lard containing benzoin to keep it from becoming rancid), and hydrous wool fat or lanolin. Vaseline is the most unchangeable of the bases, being unaffected by air, moisture or chemicals. However, it is not absorbed well, nor does it give up readily the medicinal agents incorporated in it, so that it is best suited for ointments designed for protective purposes only. Benzoinated lard is very valuable as a base as it will take up about fifteen per cent of water without losing its ointment-like consistency and in addition it readily yields up for absorption any medicinal substances which have been incorporated in it.

Wool fat is also largely used as it will take up a very considerable amount of water—even an equal weight—and

in addition it is easily absorbed. Its chief disadvantages are that it is not so easily worked as other fats and that it is quite sticky. These undesirable features may be lessened by incorporating other fats with it in different proportions.

Prepare thirty grams of belladonna ointment (*Unguentum Belladonnæ*) according to the directions given in the U. S. Pharmacopœia.

In preparing this ointment note especially the physical characteristics of the lard and the wool fat.

It is hardly necessary to say that the finished ointment should be perfectly homogeneous and free from any gritty particles.

Examine a specimen of vaseline (*Petrolatum*). Look over also the composition of other ointments than the belladonna ointment as they are described in the Pharmacopœia.

Suppositories.

Suppositories are solid medicated substances usually of a conical form and designed for introduction into the various passages of the body where they will become liquid and exercise a local action or else be absorbed and have a general or systemic effect. They consist essentially, therefore, of some drug and a base with which the medicinal substance is incorporated and which is solid at ordinary temperatures, but which liquifies slightly below the body temperature.

The base most commonly employed is cacao or cocoa butter (*Oleum Theobromatis*) which is obtained from the seeds of the plant *Theobroma cacao*, from which chocolate is obtained. The melting point of this substance lies between 86° and 95° F. Other bases which are used for special purposes are glycerin-gelatin and sodium stearate.

The size and shape of suppositories will depend upon the base employed in their manufacture, and the use to which they are to be put. If they are to be made from the oil of theobroma, the cone shaped rectal suppositories should weigh about two grammes; the pencial shaped suppository for the urethra about the same amount, and the globular suppositories for the vagina, four grammes. If on the other hand, they are made with glycerin-gelatine as a base, the weights above given must be doubled.

There are three ways of making suppositories using the oil of theobroma as a base.

In the fusion process, the oil is gently melted and the medicinal substance incorporated with it while it is still

warm and as the mass is cooling it is poured into well cooled moulds and allowed to harden.

In the cold compression method, the mixed drug and base are placed in a special apparatus and under pressure moulded into the required shape. Complete directions for these methods are to be found in the U. S. Pharmacopœia.

The third method, and the one usually employed when only a few are to be made is carried out in the following manner, which may be illustrated by filling a prescription calling for three suppositories.

R

Acidi Tannici 0.3

Olei Theobromatis 6.

Fiant suppositoria No. III.

Sig.

Use as directed.

These three suppositories are to be made by hand as follows. Put the tannic acid in a mortar and add about an equal weight of the oil, which has been grated. Mix the two thoroughly and then add the remainder of the grated oil, working the mass in the mortar until it becomes homogeneous and plastic. If necessary to make the mass cohesive, about three drops of oil of sweet almond or of glycerin may be added. Transfer the mass to the pill tile and form it into a cylinder of convenient length and cut it into three equal parts. Form each piece now into a conical suppository by means of the fingers and spatula. They should all be of the same size and shape. Dust them with a little lycopodium or starch to keep them from sticking together in the box.

Physiology of the Nervous System of the Frog.

In order to study the action of a drug upon the nervous system of a frog it need hardly be pointed out that it is of prime importance to understand the physiology of the different parts of that system. To illustrate, a certain drug will depress the cerebrum, while a second drug will stimulate the spinal cord and a third may paralyze the peripheral nerves; now, only by knowing the normal functions of these divisions of the nervous system can we recognize and interpret the symptoms arising from an increase or diminution in their activity, and thereby locate the point of action of the drug employed.

First, examine the normal frog, observing its spontaneous movements and the method of leaping and what degree of irritation is necessary to make it move. Note also its co-ordination of the muscles in swimming; its recovery of balance when slowly turned on a sloping board, its recovery when placed on its back, its reflexes when the toe is pinched, the croaking when its back and sides are stroked, the rate of respiration and heart beat. (The latter can usually be counted when the animal is turned on its back and placed in a good light, a slight shadowy movement of the skin being seen in the cardiac region with each pulsation of the organ.)

Having studied the intact animal, separate the cerebral hemispheres from the optic lobes by thrusting the point of a scalpel through the roof of the skull on a line joining the posterior borders of the eyelids, gently rocking the scalpel from side to side so as to completely sever the hemispheres from their inferior connections. As will be seen from the

figure, such a cut
by the optic thalami
symptoms will result
destruction, but for all
e, the differences in
ed are not important.
g the animal a few
cover from the effects
k of the operation
(be done in all cases)
condition with that of
animal.

notes of all changes

move the optic lobes
ne animal by cutting
with the point of a
line joining the cen-
tympenic membranes.

of the head posterior
faces about 3mm. in
re and then study the
e animal by means of
jaw and observe the
pping the toe in very
phuric acid to water).

wire down the spinal
erve any change in the
heart and respiration?

Examine the activity of the peripheral nerves in the following manner; hold the frog back upwards in the hand and apply the electrodes from the induction coil (tetanizing current) to the lumbar spine. Observe how long the muscles will stay contracted before fatigue sets in.

Dissect out the sciatic nerve by an incision along the posterior surface of the thigh and stimulate it with the electric current as before.

Lay a few crystals of sodium chloride on the nerve and moisten them with a drop of water. Observe any change in the muscles. Explain.

Expose the gastrocnemius muscle and stimulate it directly with the electric current and also with salt crystals.

Describe the functions of the different parts of the nervous system in the frog as learned from the symptoms arising from their removal.

Action of Acid Fuchsin.

In connection with the study of the physiology of the nervous system of the frog the action of acid fuchsin is very interesting and instructive. This drug when injected into frogs in proper dosage apparently produces no effects for from 12 to 24 hours. If, however, it is given in the same-sized dose to an animal suffering from fatigue or to one in which the cerebral hemispheres have been injured the symptoms will appear in about fifteen minutes.

Experiment 1. Using a 1.5% solution of acid fuchsin inject into the anterior lymph sac (see page 13) of a frog 0.3 mg. acid fushsin per gram body weight of animal. Replace the frog under the bell jar and study it from time to time, observing general activity, reflexes, etc. After 10

or 15 minutes, sever the cerebral hemispheres from the rest of the brain, according to the method described earlier, and replace the frog on the table for observation. If convulsions appear study them carefully, making adequate notes and then try to locate the point of action of the poison by cutting off the optic lobes, destroying the medulla and cord noting changes that occur after each operation.

Where does the drug act to produce the convulsions and what light does the experiment throw upon the function of the cerebrum?

Soporifics or Hypnotics.

Soporifics or *Hypnotics* are drugs which are used to produce sleep. They comprise a very large and important group of substances which are extensively employed in medicine. In their action they are closely related to chloroform and ether, but they are not used to produce anæsthesia in man owing to the fact that it is impossible to regulate sufficiently accurately the depth of the anæsthesia induced by them.

Chloral (chloral hydrate), the most important member of the series, was introduced into medicine by Liebreich in 1869, and as a pure hypnotic drug is probably the most reliable in use today. The other members of the group which are of greatest importance are *Sulphonal* (Sulphonmethane), *Paraldehyde* and *Veronal*.

Examine each drug as to appearance, odor and taste.

I. Examine the solubility of the following soporifics:

		Chloral.	Sulphonal.	Paraldehyde.	Veronal.
a	Cold water.				
b	Hot water.				
c	Alcohol.				
d	Ether.				

Wrap up a few crystals of chloral in paper, set aside and examine them after a few days.

In what ways would you think these drugs could best be prescribed in practice?

II. Put a few crystals of chloral in a test tube and add a small amount of potassium hydrate solution and heat gently. Note the odor of the fumes. What is formed?



Chloral hydrate.

Potassium formate.

III. Select three frogs of about equal size and mark each frog in some way so that it can be recognized. (This is probably done most easily by making a drawing showing the outlines of the marking on the back.) Examine the frogs, making a record of the heart rate and respiration in each.

Inject into the anterior lymph sac (page 13) of one frog 5 mg. of chloral dissolved in water. After studying this animal for about 10 minutes inject into the second frog 20 mg. of chloral. In like manner after observing this animal for a time and making notes inject into the third frog 50 mg. of chloral. These amounts may be obtained most accurately by making 5 or 10 cc. of a 5% solution and then diluting small quantities (1 cc.) so as to get the required doses.

Make a record of the time when each injection is made. After injection replace the animals under the bell jar and observe them frequently (especially those with the larger doses) as to spontaneous movements, co-ordination, reflexes, respiration, heart beat, etc.

Keep a careful record of the condition of each frog at each examination.

This can be done most systematically, not only in this experiment, but also in those to follow, by keeping a record of the time when each examination is made and the conditions found. By doing this it also gives some idea of the length of time it takes the drugs to act when given in different sized doses.

Such a record would start something as follows:

Feb. 10, '15—Frog. 23 G.

2:30—Heart 56, respiration 30 per minute.

2:32—Injected 20 mg. chloral in water into anterior lymph sac.

2:35—Frog quiet, apparently normal; reflexes, normal; heart, 54; respiration, 28.

2:40—Frog jumps if urged strongly, does not regain sitting position easily when it alights; has difficulty in turning over when placed on back. Reflexes lessened; heart, 40; respiration, 15, etc.

Such a record should give an accurate picture of the condition of the animal at each stage of the drug's action.

If complete paralysis occurs in any animal determine whether the action is on the central nervous system or is peripheral.

This is done by stimulating over the lumbar spine with the electric current and if the muscles of the leg contract the paralysis must be central; if they do not contract see whether the trouble is in the nerve or in the muscles by stimulating the latter directly with the current.

Also in one of the animals thus paralyzed, expose the heart (page 14) and examine and record its condition.

The frog given 50 mg. may be used for this purpose. The others may be replaced at the end of the day's work under the bell jar and moistened and examined on the following day. Some will doubtless recover. Notes should be kept of their progress.

With the knowledge gained of the physiology of the nervous system study the records of the three frogs and from the symptoms recorded determine the points of action of the drug in the varying doses.

IV. Inject into the anterior lymph sac of a frog 0.5 cc. of a 10% solution of paraldehyde in water. Compare the symptoms shown by this animal with those manifested by the chloral frogs in Exp. III. Pay particular attention to the condition of the heart, which can probably be sufficiently well studied (as to its rate) through the chest wall without exposing it. Keep the animal, as it will probably recover.

The difference in action of the two drugs upon the heart is explained by the difference in the constitution of the two molecules. The chloral molecule contains chlorine and these chlorine compounds all have a decided action on the heart muscle which is absent in the case of paraldehyde, whose

molecule, $C_6H_{12}O_3$, contains no chlorine. Compare the action of chloroform and ether on the heart (page 187).

Other members of this group are, Trional.

Tetronal.

Urethane.

Chloretone.

Chloralose, etc.

*Therapeutics.*¹

The chloral group of drugs depresses the central nervous system. For this purpose it would be used in cases of insomnia and of nervous excitement. Chloral and its allies are very valuable also in delirium and in various forms of convulsions, as for instance, those due to poisoning with certain drugs or those due to specific diseases (tetanus). In certain forms of insanity (mania) and in some nervous diseases with increased activity of the brain and spinal cord (chorea or St. Vitus's dance) the quieting effects of these drugs are largely made use of.

On account of its action on the heart chloral must be employed with care in persons who are already affected with certain forms of heart disease and its effect on this organ must also be watched with care in case of its prolonged use, or better, some non-chlorine containing soporific may be used in its stead.

¹ At the conclusion of the study of a drug group a short discussion of the therapeutic uses of the drugs contained therein will be given. Such discussions are not intended to give complete resumé of all the therapeutic uses of the drugs studied, but only those will be mentioned as would be indicated by the results obtained in the foregoing experiments. The student will thus be shown how the knowledge gained by the science of Pharmacology is transferred to and made use of in the Therapeutic art; or, in other words, in rational medicine.

Nux Vomica.

Nux Vomica, the seeds of *Strychnos nux vomica*, an East Indian tree, contains two alkaloids upon which the activity of the drug depends. By far the more important of the two is *Strychnine* which is present in from .9 to 2. per cent; the second alkaloid, *Brucine*, is present in slightly smaller quantities. In action the two alkaloids resemble each other very closely excepting that strychnine is about forty times as strong as brucine. For this reason the activity of nux vomica may be considered as dependent upon the amount of strychnine present and in the following experiments it alone will be considered.

Examine the nux vomica bean and taste a little of the powdered drug.

I. Place a small amount of the powdered drug in a beaker and pour upon it 10 cc. of boiling water and let it stand until cold. Set this water extract (infusion) aside and use it in experiments IV and V given below. Place a drop of the infusion on the tongue.

II. Prepare a fluid extract of nux vomica in the following manner:

Nux vomica, No. 60 powder—50 grams.

Alcohol and water in the proportion of 2:1.

Moisten the powder with 25 cc. of the diluted alcohol and let it macerate in a well covered vessel in a warm place for 48 hours. Pack it in a percolator (see percolation, page 44) and gradually pour on diluted alcohol until the drug is exhausted. Reserve the first 35 cc. of the percolate and evaporate the remainder over a water bath to a soft extract. Dis-

solve this extract in the 35 cc. saved and make the total bulk up to 50 cc. with diluted alcohol.

Define a fluid extract.

III. Isolate the strychnine in the fluid extract in following manner:—

Dilute the fluid extract with about twice its own volume of distilled water and pour it into a separating funnel and render it alkaline with ammonia. Add about 25 cc. of a mixture of chloroform and ether in the proportion of 2:1 and shake gently for three minutes and set the funnel aside to allow the chloroformic solution to separate. When separation has taken place, draw the chloroform and ether off into a beaker and repeat the agitation of the watery extract, using a fresh supply of chloroform and ether. Draw this off as before into the breaker with the first solution. The watery extract can now be thrown away. Pour the chloroform and ether into the separating funnel, which has been washed, and add about 25 cc. of water and enough dilute H_2SO_4 to make the solution distinctly acid, shake gently for a few minutes and then separate the water and chloroform as before, placing the acidulated water in a flask. Repeat the agitation of the chloroform and ether with fresh acidulated water, adding the latter when separation has taken place, to the first lot in the flask. (The waste chloroform is to be poured into a large bottle provided for it and later redistilled.) As the acidulated watery solution is probably colored it should be shaken in the flask with charcoal and filtered. Render the filtrate distinctly alkaline with ammonia and shake it out twice as before in the separating funnel, with small quantities of the mixture of chloroform and ether. When this has been done, pour this chloroformic

solution into a glass evaporating dish, add to it a drop of concentrated hydrochloric acid and stir it well with a glass rod or put the mixture in a small flask and shake it well. The solution becomes milky from the formation of strychnine hydrochloride, which is thrown out of solution and may be separated from the chloroform by allowing the latter to evaporate spontaneously or by warming it gently over a water bath.

CAUTION.—In this process two things are of prime importance in determining the success of the manipulations:—First, to agitate sufficiently the chloroform and ammonia or sulphuric acid solutions to allow thorough intermingling of the solutions. Three to five minutes should be devoted to each shaking. Second, make sure that the correct reaction is present. Test with litmus paper each time and after shaking for a minute or two, test again. (See Alkaloids, Exp. No. II.) The reaction must be distinct each time.

Explain why each step in the manipulation was carried out. (Compare Alkaloids, Exp. Nos. I, II, VII.)

The crystallized strychnine hydrochloride is to be used in tests given below. (Exp. Nos. X, XI.)

IV. To a few cubic centimeters of the infusion made in Exp. I add a few drops of ferric chloride solution. The dark color of the mixture is due to the tannic acid present in the nux vomica bean uniting with the iron to form ferric tannate. (To a dilute solution of tannic acid add ferric chloride.)

V. Inject 0.5 cc. of the infusion into the lymph sac of a frog, and replacing the animal under a bell jar, observe the effect. Keep full notes. If any convulsions come on, after

studying them carefully, try to find out the point of action of the drug by dividing off in turn the cerebrum, optic lobes, and finally destroying the cord, watching the effect of each operation on the symptoms. The strychnine convulsion is "tonic" as distinguished from the "clonic" type, which is caused by some drugs and occurs in some diseases. (Compare Camphor(page 112.)

Make a drawing of a frog in a strychnine (tonic) convulsion.

VI. Inject into the lymph sac of a second frog $\frac{1}{4}$ cc. of a 1/10% solution of strychnine sulphate diluted with physiological salt solution to make a convenient amount. In this animal, as in the previous experiment, study the early effects of the poison before convulsions come on. Look for changes in irritability, in reflexes, etc. When convulsions come on, paint the skin of the animal with a 2% solution of cocaine observing any changes which occur. If the convulsions cease wash the animal off with water and put it to one side observing it again at the end of 10 or 15 minutes. Explain.

If paralysis should occur in any of the frogs used in these experiments see if it is due to central or peripheral action, as was done in chloral, Exp. III.

VII. Inject into a small frog 1/20 mg. of strychnine. When convulsions come on place the animal under a bell jar with some absorbent cotton upon which has been poured some chloroform. Observe whether any change takes place in the convulsions. If they become lessened, remove the chloroform from the bell jar and allow the animal to recover from its effects. Do the convulsions return? When they do, pith (page 13) the brain of the animal and hang it up by the jaw on the hook for reflexes. Now test the animal's reflexes

by pinching the toe and by dipping the foot in acid. How do they differ from those in a normal animal?

Is any therapeutic hint contained in the first part of this experiment? In experiments Nos. V, VI and VII, it was noticed that the same effects were induced whether the poison was employed as the infusion, or as the pure alkaloid.

VIII.¹ The object of this experiment is to apply strychnine to the cervical portion of the cord while leaving the lumbar cord unaffected. Instead of pithing a frog as usual, remove the brain entirely by cutting off the head with a pair of scissors. Put the lower blade in the mouth and the upper blade over the top of the head, incline the scissors backward slightly and make a quick cut. Expose the heart and remove it from the body in order to stop the circulation. The upper part of the cord will be exposed by the cut first made and a drop of a moderately strong strychnine solution (0.1%) is to be applied to it. After about five minutes test the reflexes by pinching the fore and hind legs. If the experiment is successful a normal reflex should be obtained by pinching the toes of the hind foot, while a typical strychnine convulsion involving the entire animal should be brought on by irritation of the fore limbs.

How would such results help to locate the point of strychnine activity?

(See Pharmacology and Therapeutics, Cushny. Refer to chapter on Strychnine.)

IX. With a hypodermic syringe inject subcutaneously into a rabbit 1 mg. of strychnine sulphate. Watch the effect

¹ This experiment is not always successful when carried out by students and may be omitted if thought best. However, if successful, it is of so much interest and importance in locating the point of action of the drug as to merit a place in these notes.

on reflexes, etc. If convulsions come on control them with a few drops of chloroform on cotton held close to the animal's nose.

Compare the convulsions in this animal with those seen in the frog. Note the efficiency of chloroform in controlling the convulsions of strychnine.

Chemical Tests for Strychnine.

Xa. Put one or two crystals of the strychnine hydrochloride, prepared in Exp. III, on a porcelain cover and add to it two drops of concentrated sulphuric acid, mixing the two with a clean dry glass stirring rod. Put beside the strychnine-sulphuric acid mixture on the cover a minute crystal of potassium bichromate and with the glass rod (which has been washed and dried) draw the bichromate crystal through the strychnine mixture. Observe the changes in color. This "oxidation-reaction" or "fading purple test" for strychnine is most delicate and characteristic. It is said to be capable of detecting 1/20000th of a grain of the alkaloid. The order of the color changes is very important in making the test. The purple, which should first appear, changes to a crimson, which turns to a cherry red that is fairly persistent.

Xb. Carry out the same experiment as above, using manganese dioxide instead of potassium bichromate. Compare the results with Exp. Xa.

This reaction is obtained with almost any oxidizing agent, such as potassium permanganate, potassium ferricyanide, or lead dioxide. Manganese dioxide is one of the best as the changes in color take place very slowly, in some cases lasting some minutes.

If much brucine is present in the preparation, this color test is much interfered with and it may be impossible to get the correct changes.

Xc. After testing your own alkaloid with the two reagents named, make the same tests on some pure strychnine which will be furnished you. Compare the colors with those obtained with your preparation.

XI. Dissolve the rest of the strychnine hydrochloride (Exp. III) in about 20 cc. distilled water and put the solution into 5 test tubes, diluting with a little water if necessary and test them with the various alkaloidal reagents.

	Reagent	Precipitate?	Limit of delicacy of reagent
a	Tannic acid.		
b	Picric acid.		1-20,000
c	Iodine in potassium iodide.		1-100,000
d	Mercury-potassium iodide.		1-150,000
e	Phosphotungstic acid.		1-200,000

Therapeutic uses.

On account of their intensely bitter taste, preparations of *nux vomica* are employed in cases of loss of appetite and malnutrition. By their action on the taste organs they probably increase reflexly the secretion of the gastric juice and thus aid digestion.

Strychnine has been largely used in the past as a stimulant to the central nervous system in various conditions in which depression of the brain or cord was present. It was employed in shock or collapse, and in failure of the respiratory or of the vaso-motor center. In recent years considerable

doubt has been expressed as to whether the drug exerts any beneficial action in such conditions when administered to man in therapeutic doses. This doubt has led to the restriction of its use but it is still employed to a certain extent. By stimulating the activity of the spinal cord to a slight extent it increases the "tone" of the muscles and of the blood vessels whose muscular coats are controlled by the spinal centers.

Camphor.

Camphor is a white, translucent, crystalline substance obtained from the volatile oil of the camphor tree (*Cinnamomum Camphora*). In addition to its local irritant action, it exerts a very marked effect upon the central nervous system producing symptoms which are quite characteristic and which may well be studied in connection with those produced in mammals by strychnine.

I. Weigh a cat or rabbit and then administer to it through a stomach tube camphor dissolved in olive oil (2 G. camphor for each kilo of body weight). Replace the animal in a cage and observe the early symptoms and the convulsions which should come on in about half an hour. Compare them carefully with those produced by strychnine and try to control them with chloroform. (After observing them sufficiently the animal should be killed with the anæsthetic.)

A similar convulsion is also caused by thujon which is an isomere of camphor and the active poisonous principle derived from the volatile oil of sage, tansy, and absinth. It may be used in place of the camphor in the above experiment if desired. It is administered to cats by means of the stomach tube in doses of about 2 cc. (Some preparations of thujon are active in 1 cc. doses while others require 5 cc.) Note the marked change in respiration which occurs before the convulsions come on.

It will be seen that the convulsions caused by thujon are also of a distinctly different type from those following a poisonous dose of strychnine. Those caused by the latter drug are tonic in type and are due to an action on the spinal

cord. On the other hand, the thujon (and camphor) convulsions are "clonic" and are caused by stimulation of some unknown part of the cerebrum or lower part of the brain.

Picrotoxin, the active principle of a climbing shrub, *Anamirta paniculata*, indigenous to India, also has a pharmacological action much like the drugs mentioned above, and the clonic type of convulsion may be very easily demonstrated by its use.

II. Inject into a frog 1 mg. picrotoxin and replace the animal under the bell jar. When the convulsions come on study them carefully indicating by a drawing the position the animal takes. Remove in order the different portions of the central nervous system by successive cuts as has been done in previous experiments and thus determine the point of action of the drug.

III. Inject subcutaneously into a guinea pig 5 mg. of picrotoxin. Study the convulsions as before.

Thujon and picrotoxin are not employed in therapeutics. Their pharmacological action would indicate that they might be of value as stimulants to the central nervous system and especially to the respiratory center. As such they might be of value in shock and collapse and perhaps in narcotic poisoning. Camphor has long been used in shock and collapse.

Opium.

Opium is the inspissated juice obtained from the fruit-capsules of the white poppy (*Papaver somniferum*). The capsules are incised while yet unripe and the milky juice allowed to exude and dry by spontaneous evaporation until it becomes a brownish gummy mass.

The poppy is grown in Turkey, Asia Minor, Persia, India and China, but that obtained from Smyrna and Turkey is by far the most valuable. Persian and Indian opium is imported mainly as sources of the opium alkaloids and the Chinese opium is consumed entirely in that country.

Opium contains about twenty alkaloids of which *Morphine* and *Codeine* are the most important, and, indeed, it may be said that the value of opium depends upon the per cent of morphine which it contains. The U. S. Pharmacopœia specifies that the crude opium when moist shall contain not less than 9.5 per cent of crystallizable morphine, while the dried product must assay from 10 to 10.5 per cent. Opium from the different localities varies exceedingly in its morphine content.

Morphine was discovered in 1817 by Seitürner, of Eimbeck, Germany, and was named by him morphium. It exists in the opium principally in combination with meconic acid, although some may be present as a lactate or a sulphate, both of these acids being found in the crude drug. Meconic acid is of most importance as it is strictly characteristic of opium and its preparations, and its positive detection is proof of the presence of some opium preparation.

In their solubilities morphine and codeine differ widely from the other alkaloids, for morphine is relatively insoluble

in chloroform and even less soluble in ether (1-6250); codeine, on the other hand, is more soluble in water than any other alkaloid except caffeine.

Morphine Reactions.

I. Put a few crystals of morphine on a porcelain cover and add a drop or two of pure concentrated sulphuric acid and mix the two with a clean dry glass rod. No color should be formed if the morphine is absolutely pure, but occasionally a faint pink tint is seen. Heat the mixture very gently. At about 150° a dirty green or rose-red color is developed, and on still further heating, the solution becomes almost black. Allow it to cool and add some water, when a greenish blue color appears which changes to green when ammonia is added in excess.

II. Put a few crystals of morphine on a cover and mix them with a drop or two of concentrated sulphuric acid as in Exp. I. To this add a drop of distilled water which will heat the mixture. Now treat with a drop of concentrated nitric acid, which should give a rose-red color, changing to brown.

(This reaction for morphine is very delicate, being well shown by 0.01 milligram of the alkaloid.)

III. To a drop of concentrated nitric acid on a porcelain cover add a minute amount of morphine. An orange-red color is obtained, which changes to yellow on heating.

IV. (a) Mix a small amount of morphine with about twice its weight of cane sugar. By means of a glass rod add a drop of concentrated sulphuric acid. This should produce a purple color, changing gradually to blood-red and brownish-red and becoming an olive-brown on dilution with water.

(b) The above test can be modified as follows:—To a solution of morphine add cane sugar until the solution is saturated. Now pour this solution carefully down the side of a second test tube in the bottom of which is concentrated sulphuric acid. By inclining the second tube the solution can be poured in so that no mixture of the two fluids takes place and a purple or rose-red ring will form at their junction. This ring becomes more intense on standing.

V. Dissolve some morphine sulphate in a little water and add it to a solution of iodic acid. What color does it turn? Add to the resulting mixture some thin starch paste. What was the first color due to?

NOTE.—In order to color the starch paste blue morphine must be present in the strength of 1 to 1000. If no blue is obtained mix the solution well and pour down the side of the test tube some very dilute ammonia. A double ring will be seen at the junction of the two fluids; it will be blue below and brown above.

VI. To a few cubic centimeters of water add some morphine sulphate and to this solution add a few drops of ferric chloride solution. A deep blue color is obtained.

VII. Meconic acid reaction. To a cubic centimeter of tincture of opium diluted with a little water add two drops of ferric chloride solution. What color does the solution become? If the color is obscured by a precipitate, filter the solution.

VIII. Inject into the anterior lymph sac of a frog 15 mg of morphine sulphate. Replace the animal under the bell jar and examine it from time to time, comparing the effects with those caused by chloral or strychnine, and with those seen in the frog injected with codeine in Exp. IX.

NOTE.—This experiment (VIII) should be begun early in the laboratory session as it sometimes takes two or three hours for the full effects to appear.

IX. Inject in the anterior lymph sac of a frog 1 cc. of a 1.5% solution of codeine phosphate and replace the animal under the bell jar. Compare the symptoms in this animal with those seen in the morphine frog noting especially the relative time of onset of the symptoms in the two.

Among the alkaloids found in opium which are of less importance than those studied is thebaine, which resembles strychnine very closely in its pharmacological action. In fact, many of the alkaloids of opium may be arranged in a series with the depressant, morphine, at one end of the series and thebaine at the opposite end. A comparison of the effects of thebaine with those produced by morphine and codeine may be made by injecting 1 cc. of a 1.5% solution of thebaine into a frog and comparing it with the animals studied in Exp. VIII and IX.

X. Inject subcutaneously into a dog 50 mg. of morphine sulphate and observe the effects keeping an accurate protocol of the symptoms.

XIa. Inject 30 to 40 mg. of morphine sulphate subcutaneously into a cat. Put the animal in a wire cage and compare the effects with those seen in the dog. (Note under Exp. VIII applies here also.)

XIb. Instead of injecting a cat a dose of from 5 to 10 mg. of morphine sulphate may be given a white mouse. Symptoms much like those produced in the cat are seen, the main difference being that they appear in from 5 to 10 minutes.

The peculiar action of morphine on the cat is not confined to that animal, but is found in all the members of the cat tribe, and also in the horse and ass.

- Examine
- a. crude opium,
 - b. powdered opium,
 - c. extract of opium,
 - d. tincture of opium (Laudanum),
 - e. camphorated tincture of opium (Paregoric),
 - f. powdered opium and ipecac (Dover's powder),
 - g. codeine,
 - h. and heroine.

Note the odor of all the opium preparations and taste d, e, and f.

Therapeutics.

The symptoms induced by opium in man resemble more closely those in the dog than those in the other animals. It depresses the brain, and for this reason is used in cases of sleeplessness when this condition results from pain, because in depressing the cerebrum it acts on the pain centers, for which it seems to have a special affinity. In the dog the respiration was slowed in the later stages of the action from depression of the respiratory center, and opium preparations are often employed in cough mixtures to allay excessive irritability of this part of the medulla. The numerous other purposes for which opium and morphine are used cannot be illustrated experimentally.

Curara.

Curara, the South American arrow poison, varies in composition with the different localities from which it is obtained. The best known product comes from Guiana and is prepared from the bark of *Strychnos toxifera*, a tree native to that country. Curara as obtained in commerce consists of dark brown, shining, brittle masses which contain vegetable extracts in addition to two alkaloids, tubocurarine and curine. The characteristic action of the drug is dependent principally upon the first alkaloid mentioned, the latter having quite a different action and being much weaker.

Not only do solutions of curara¹ deteriorate on keeping, but, what is of greater importance, the crude drug, as obtained in the market, varies greatly in strength, many of the preparations being entirely inert. There is no chemical method by which the activity of curara can be estimated; the only test is pharmacological. Each solution to be used has to be "standardized", by tests on animals and the dose necessary to produce characteristic effects ascertained.

I. Inject into a frog a poisonous dose of a standardized solution of curara. Study the effect on spontaneous movements, reflexes, ability to turn over when placed on its back, and length of time leg muscles will remain contracted when stimulated by applying the tetanizing current to the lumbar spine. If paralysis should occur see if it is due to central or peripheral action (see Soporifics, Exp. III, page 92). This animal is to be replaced under the bell jar and kept moist, as it will probably recover in a few days.

¹ The curara solution should be made up in physiological salt solution to which some thymol has been added.

II. Pith a frog, tie it on the frog board and expose the sciatic nerve and artery along the back of the thigh by a short longitudinal incision through the skin, and careful separation of the muscles with the dissecting needle. Separate the sciatic nerve from the surrounding tissues along a short distance of its course and then pass a thread under the nerve and tie it tightly around the rest of the thigh as high up as possible.

Free the animal, inject curara as in I, and from time to time compare the reflexes in the hind legs. Stimulate the lumbar cord with the electric current and note any differences between the two sides. See whether the reflexes remain equal in the two legs and whether cross reflexes can be obtained.

If the experiment is successful the operated leg should not be affected by the poison and should respond to electrical stimulation. In such a case cut off the leg just above the knee, leaving attached to it the sciatic nerve which has been carefully separated from the thigh muscles and cut toward the upper part of its course. Holding the leg preparation by the severed end of the femur lay the hanging sciatic nerve for a few moments in the curara solution. Remove it and stimulate it with the electric current. Does curara affect the nerve trunk? Dip the muscle in the solution and after a few moments stimulate the sciatic nerve again. Is there any change? If so, test the activity of the muscles by direct stimulation. Where does curara act?

Examine the heart of the animal in Exp. II.

Examine *Curara*.

Therapeutics.

The main importance of curara is in physiological experiments. It has been suggested, and in some few cases used, to allay the convulsions due to tetanus or to strychnine poisoning. It is not recommended for such conditions as its action is too uncertain and too dangerous.

Nicotine.

The alkaloid, nicotine, is obtained from the dried leaf of the tobacco plant (*Nicotianum Tabacum*) in which it is found to the extent of from 2 to 8 per cent. The pure alkaloid is a colorless, oily fluid, which, on exposure to air, becomes yellowish or brown. It has a strong, unpleasant odor resembling somewhat that of tobacco.

In an aqueous solution the alkaloid gives a strong alkaline reaction and it unites with various acids to form salts.

I. Inject 0.5 cc. of nicotine chloride solution¹ into the anterior lymph sac of a frog. Observe the effects, noting especially the posture of the animal in the early stages of poisoning as this is very characteristic of nicotine action. In addition, watch for any twitching of the muscles, and if paralysis should come on see whether it is due to central or peripheral action.

Make a drawing of the animal in the early stage of the nicotine action when the legs are drawn up over its back.

The action of nicotine upon the sympathetic nervous system is studied later (pages 248, 260).

Therapeutics.

Nicotine is not employed in therapeutics.

¹ The nicotine chloride solution is formed by neutralizing a small quantity of nicotine with 1-20 normal hydrochloric acid. Each cubic centimeter will then contain about 4 mgs. of nicotine.

Veratrine.

Veratrine is an alkaloid prepared from the seeds of *Asa-groea officinalis*. It is a grayish powder which causes very marked irritation when even a minute quantity is inhaled.

The alkaloid is not of great medical importance but is of pharmacological interest, owing to a peculiar action it possesses which can be best studied in cold-blood animals, although the same effect may be elicited in mammals.

I. Inject 1 mg. of veratrine sulphate (1 cc. of 0.1% sol.) into a frog. Watch and describe the effects. Observe the awkwardness of its movements which appears in a few minutes. When the clumsiness is well developed locate the point at which the drug acts by removing the cerebrum, optic lobes, and finally destroying the spinal cord if the symptoms do not disappear earlier. Stimulate the sciatic plexus with single shocks from the induction coil, comparing the results with those seen in a normal frog. Repeat the stimulation of the sciatic nerve several times at short intervals studying the effect of fatigue upon the veratrinized muscle. Finally, after allowing the muscle to rest, stimulate the muscle directly, using single shocks. At what point does the drug act?

Therapeutics.

The phenomena seen in this experiment give no hint as to the use of the drug in therapeutics; it is employed for external application, and internally to a limited extent for its effects on the circulation.

Caffeine.

Caffeine differs from the other alkaloids in the fact that it is contained in a number of plants belonging to different species. It is obtained from the berry of the coffee (*Coffea Arabica*); from the leaves of tea (*Thea Chinensis*), besides being present in the kola nut of Central Africa; in the Paraguay tea and Guarana paste of South America and in the Apalache tea of Virginia and Carolina.

In some of these plants it is associated with two closely related alkaloids, *Theobromine* and *Theophyllin*, which resemble caffeine very much in some of their actions. All three alkaloids are derivatives of xanthine, which in turn is closely related to uric acid.

Caffeine is a very feeble base and forms salts only with difficulty and these salts, as a rule, are very unstable, being easily decomposed, even by water.

I. Test the solubility of caffeine in

	Reagent.	Solubility.
a	Water, cold.	
b	" hot.	
c	Alcohol.	
d	Chloroform.	

II. Put 100 mg. caffeine in a test tube and add 4 cc. cold water to it. Shake. Now add 100 mg. sodium benzoate and shake. What therapeutic use could be made of this reaction?

III. Compare in like manner the solubility of theobromine with that of the double salt, theobromine sodium salicylate, and note the advantage the latter might possess.

IV. Apply the various alkaloidal reagents to a 1% solution of caffeine.

	Reagent.	Precipitation?
a	Tannic acid.	
b	Picric acid.	
c	Iodine in potassium iodide.	
d	Mercury-potassium iodide.	
e	Phosphotungstic acid.	

NOTE.—In its reaction to c and d caffeine differs from all other alkaloids except theobromine and colchicine.

V. Murexoin test. To a few crystals of caffeine on the porcelain cover add a drop of concentrated nitric acid. Hold the cover with a pair of forceps some distance above the flame of a Bunsen burner and thus gently evaporate the mixture to dryness. After cooling the porcelain cover allow the residue to come in contact with strong ammonia fumes and note the color obtained.

This reaction is given by the members of the xanthine series and is practically identical with that given by uric acid.

VI. Inject 5 mg. of caffeine (1 cc. 0.5% sol.) into a frog. Study and describe the symptoms comparing them with those induced by strychnine and veratrine. See whether the peculiar change in the muscles is due to an action on the nervous system by destroying its various parts as was done with veratrine. Excise a small piece of the affected muscle and tease it in salt solution on a microscopic slide. Examine it under the microscope, using the low power lens, and compare its appearance with normal muscle prepared in the same way. (For an explanation of the changes consult Pharmacology and Therapeutics, Chapter on Caffeine.)

The action of caffeine and its allies on the heart and on the kidney will be studied later (pages 207, 240).

Examine the Coffee bean (*Coffea Arabica*);
Tea leaves (*Thea Chinensis*);
Caffeine citrate;
Theobromine.
Theobromine sodium salicylate.

Therapeutic Uses.

Caffeine is largely employed as a central nervous stimulant. The stimulant action on the highest parts of the brain is more noticeable in man than in the frog as the cerebrum in the latter is not as highly differentiated and the principal symptoms are due to cord action. In man, however, caffeine (usually taken in the form of coffee or tea) stimulates the cerebrum and the medulla and, to a less extent, the spinal cord. It is of great use in cerebral depression, such as is found in shock and collapse. In narcotic poisoning, also, its action on the medullary centers is especially valuable. Both caffeine and theobromine are largely used for their stimulant action on the kidneys by which they increase the urine. They are, therefore, of service in removing abnormal collections of fluid from the body, such as œdema or ascites, whether of cardiac or renal origin.

Cocaine.

Cocaine is the principal alkaloid derived from the leaves of *Erythroxylon coca*, a shrub which is a native of Peru and Bolivia and of other parts of South America. It is very unstable, being quite prone to undergo chemical change, so that preparations of the drug frequently contain the products of its decomposition. For this reason there are no very delicate or distinctive chemical tests by which the drug may be identified. Being an alkaloid, it is thrown out of solution by the various precipitants of the group, and in its behavior to solvents it resembles in general the other alkaloids.

Cocaine has an important local action upon the terminations of sensory nerves, which makes it of very great use in medicine.

I. Take a piece of filter paper 2 or 3 cm. square and moisten it with a few drops of a 4% cocaine hydrochloride solution and place it upon the tip of the tongue. Retain it in this position for two or three minutes and then examine the condition of that part of the tongue exposed to the drug, testing it especially as to the sense of touch, temperature, and taste. Contrast its effect on the taste of sugar and of nuxvomica.

II. Place a cat in a cat box allowing the head to protrude. After examining the corneal reflex and the reaction of the iris to bright and dim light, put into one eye 2 drops of a 4% solution of cocaine hydrochloride, keeping the other eye as a control. After 5 minutes examine the eye testing the reflexes to touch and to both dim and bright light and comparing the size of the pupil with the control. If

the drug effects are not sufficiently well marked make a second or even third application of cocaine allowing 5 or 10 minute intervals to elapse to allow of absorption. What therapeutic uses may be made of these actions of cocaine?

In addition to these local actions cocaine has also a marked effect on the central nervous system which may be illustrated by the following experiment:

III. Inject subcutaneously into a white mouse¹ from 3 to 5 mg. of cocaine. Place the animal in a suitable container and observe.

Therapeutic Uses.

Cocaine is most largely employed in medicine for its action in paralyzing the sensory nerve endings causing a local anæsthesia, permitting of operations being performed without pain. It is also employed to dilate the iris in order that examinations of the interior of the eye may be made. It is rarely used for its action on the nervous system.

¹ Somewhat similar symptoms may be obtained in the dog by the injection of cocaine in doses of from 20 to 25 mg. per kg. If convulsions come on they may be controlled with chloroform.

The Belladonna Series.

Several plants belonging to the Solanaceæ furnish alkaloids which are very closely related, not only chemically, but also in their pharmacological effects. Chief among these alkaloids is *Atropine* which is contained in the leaves and roots of the deadly nightshade (*Atropa Belladonna*). Next in importance to atropine are *Hyoscyamine* and *Hyoscine*. These alkaloids are all obtained from other sources than that named, viz.; henbane (*Hyoscyamus niger*), the thorn-apple (*Datura Stramonium*) and other plants of less importance. They are very hard to isolate in absolutely pure form because hyoscyamine is very prone to change to atropine during the manipulations.

As stated above, they resemble one another closely in their properties and actions, so that atropine alone will be studied. Examine Belladonna leaves and roots.

I. Prepare the alcoholic extract of belladonna leaves according to the U. S. P., using 50 G. of the powdered drug.

Define an "extract" of a drug.

II. Take some of the extract, an amount about the size of a pea and rub it up in 5 cc. of salt solution and put a drop of the watery extract in a cat's eye and watch for any changes comparing it with the normal eye. The cat is best put in a cat box (footnote, page 12) with the head exposed during the experiment; drop the solution near the outer canthus of the eye so that it will have to cross the eyeball before escaping by the nasal duct.

This action of the atropine series upon the pupil is known as mydriasis and the drugs producing it are mydriatics. It

is the most delicate test known for the group, although a few other drugs, of which cocaine is the most important, act as mydriatics, though to a less marked degree.

III. Isolate the atropine from the extract of belladonna by the "shaking-out" process as described below. Observe the precautions mentioned under the isolation of strychnine (page 100).

Mix the extract thoroughly with about 100 cc. of distilled water and render it alkaline with ammonia. Put the mixture in a separating funnel and add 20 cc. of chloroform. Shake gently for 3 to 5 minutes and set the funnel aside to allow the chloroform to separate, drawing it off into a flask. Add 15 cc. more chloroform to the watery extract and shake as before, and draw off the chloroform into the flask with the first lot. The watery extract can now be thrown away.

Put the chloroform containing the alkaloid in the separating funnel and add about 50 cc. of water rendered distinctly acid with sulphuric acid. Agitate the mixture several minutes as before, drawing off the chloroform from the water when separation has taken place. Repeat the process with the chloroform and 50 cc. of fresh acidulated water. (The waste chloroform is to be placed in a jar on the side table for redistillation.) If the watery solution is colored, even slightly, shake it in a flask with powdered charcoal and filter it. The filtrate containing the atropine is now rendered alkaline with ammonia and shaken out again with 20 cc. of chloroform, which is drawn off into a glass evaporating dish. Repeat the process with 10 cc. of chloroform and add it to the 20 cc. in the dish and set the whole amount aside, protected from the dust, to allow the chloroform to evaporate and the atropine to crystallize.

IV. Place a few crystals of atropine (from Exp. III) on a porcelain cover and add to them a drop of concentrated nitric acid and evaporate it to dryness over the water bath. Allow the cover to cool and then touch the residue with a glass rod which has been dipped in an alcoholic solution of caustic potash. A rich violet color is produced, changing to a dark red, which finally fades away, but can be reproduced by the addition of fresh alcoholic potash. This reaction (known as Vitali's test) is almost peculiar to the atropine series and is said to be given by 0.0001 mg. of the alkaloid.

V. Inject into a frog 15 mg. of atropine sulphate and describe all the symptoms which are observed following the injection of the drug. Keep the animal for several days until complete recovery takes place. Compare the effects with those induced by other drugs used earlier in the course.

VI. Count the heart and respiratory rates in a dog which is resting quietly and then inject hypodermically 1 mg. atropine sulphate per Kg. body weight. After the drug has had time to be absorbed repeat the observations previously made and if necessary take further counts later keeping a record of the findings. Explain the results.

VII. Take 0.001 G. of atropine sulphate (which will be furnished you) and describe all the symptoms experienced. Count the rate of your heart before and after taking the drug and see if there is any change.

VIII. Dissolve the remainder of the atropine isolated in about 20 cc. of distilled water which has been rendered acid with sulphuric or hydrochloric acid. Divide the solution into five parts, put it in test tubes and add the alkaloidal precipitants as follows:

	Reagent.	Precipitate?
a	Tannic acid.	
b	Picric acid.	
c	Iodine in potassium iodide.	
d	Mercury-potassium iodide.	
e	Phosphotungstic acid.	

The action of atropine on the innervation of the heart is studied on the turtle (page 196); and on the frog (page 187).

Its action on secretions will be studied on the salivary gland (page 263).

Its mydriatic effect when applied locally was shown in Exp. -II, and the same effect from internal administration is studied under the heading "Cervical Sympathetic Nerves," (page 251).

Chemically atropine consists of a combination of tropine with tropic acid. Other alkaloids have been made artificially by the substitution of other acids in the place of tropic acid. The most important of these synthetic alkaloids is Homatropine in which tropine is combined with mandelic acid. In many of its actions it resembles atropine quite closely, the main point of difference being that its effects are much more transient. Its main use is in ophthalmology where it is employed to dilate the iris and paralyze the accommodation by acting on the ciliary body.

Examine *Atropa Belladonna*,
Hyoscyamus niger,
Datura Stramonium,
the alcoholic extract of belladonna leaves,
tincture of belladonna leaves and
the extract and tincture of hyoscyamus.
Homatropine hydrobromide.

Therapeutic Uses.

Owing to the fact that atropine has such a wide range of activities, its uses in medicine are very numerous. Among these a few may be mentioned as being indicated by the various experiments in which the drug is employed. It is used as a stimulant in the depressed state of the central nervous system occurring in shock, collapse and in narcotic poisoning. (Compare strychnine and caffeine.) For its peripheral action it is employed for many purposes. In cases of extreme slowness of the heart (Bradycardia) due to overactivity of the vagus, atropine may be employed. It is used to dilate the pupil of the eye to allow of ophthalmoscopic examination, or as a therapeutic agent in the treatment of many eye diseases.

In excessive secretion of the saliva such as occurs in poisoning with mercury, and in the night sweats of tuberculosis, atropine is very valuable.

Finally it is used in cases of poisoning by pilocarpine or muscarine, the latter being an alkaloid resembling pilocarpine in many of its actions.

Cinchona.

In the plants belonging to the cinchona family are a number of species the bark of which yields some twenty-five alkaloids. *Quinine* is by far the most important of these. The Cinchonacæ are natives of South America and, as far as is known, were introduced into medicine about 1630.

Quinine may be regarded as a typical alkaloid and its behavior towards various solvents and precipitants has already been examined (page 20).

In addition to those properties, which it shares in common with the other alkaloids, it shows a few reactions of its own which are more or less characteristic.

Taste quinine and quinine hydrochloride.

I. Weigh out 0.1 G. quinine, the same amount of quinine sulphate, and of quinine hydrochloride and put them separately in three test tubes. Add 10 cc. of water to each test tube and shake. Which is the most soluble?

II. To 0.1 G. quinine sulphate in a test tube, add about 10 cc. distilled water and about 1 cc. of dilute sulphuric acid. Note the change in solubility compared with I and describe the solution formed. Solutions showing this peculiar fluorescence are also formed by a few of the other cinchona alkaloids.

III. Make a solution of quinine sulphate in a test tube, adding a *drop* or *two* of sulphuric acid if necessary. Add very carefully potassium hydroxide to make the solution as nearly neutral as possible (not alkaline!). Add a few drops of chlorine water and after shaking add ammonia to excess.

Observe the greenish coloration of the solution or the green precipitate which will be formed if enough quinine is present.

This reaction is of importance, as it can be employed in testing for quinine in the urine in cases in which it is important to know that the drug is being absorbed.

Examine Cinchona bark.

Tincture of cinchona.

Compound tincture of cinchona.

Quinine tannate (Tasteless quinine).

Euquinine (Quinine ethyl carbonate).

Therapeutic Uses.

The tinctures of cinchona are largely employed in medicine on account of their bitter taste; they are given especially in cases of lack of appetite. The other therapeutic uses of cinchona and its alkaloids cannot be demonstrated very satisfactorily in the laboratory.

The tannate of quinine and euquinine may be used in case tasteless preparations are desirable as in children.

Anaesthetics.

The most important members of the group of drugs used to produce general anæsthesia are *Chloroform* and *Ether*. Their depressant action on the central nervous system will be observed in some of the experiments in which they will be used to supplement the other drugs used as anæsthetics in animals (chlorotone, etc.). The cardiac effects are studied in the frog, turtle and dog. In these animals it will be noticed that the heart dilates and becomes very weak before there is very much change in the rate. The importance of this in practical anæsthesia is that information as to the condition of the heart cannot be derived entirely from the pulse rate, as the heart may be seriously affected and yet show very little change in the number of its contractions per minute. The quality of the pulse must also be noted.

The fact that chloroform acts very much more strongly on the heart than ether, will also be demonstrated in the experiments mentioned above.

Some of the early effects of the anæsthetics are shown in the following experiment:—

I. Place a rabbit on the table and hold it by placing your hand under its chest in such a way that you can count its heart and respiration. Pour some chloroform on a little absorbent cotton and hold it to the animal's nose for a few moments and observe any change in the rhythm of the heart or respiration.

Repeat the experiment using ether and afterwards ammonia. It will be seen that the same effects are produced by all three drugs. They are caused by a reflex stimulation of

the vagus and respiratory centers from the action of the irritant fumes on the mucous membranes:

Other drugs that are used as general anæsthetics are *Nitrous oxide gas* and *Ethyl chloride*.

Nitrous Oxide.

The action of nitrous oxide may be observed by placing a frog, white mouse, or small guinea pig in a wide mouth bottle which is fitted with a cork through which pass two pieces of glass tubing. One of these tubes is connected with a tank of nitrous oxide gas. When the animal has been placed in the bottle and the cork adjusted, nitrous oxide gas may be admitted by opening the valve very gradually. The outlet glass tube should be left open.

Observe the animal closely as the percentage of gas increases in the jar, noting especially changes in the rate of respiration. Keep a record of the time it takes to produce complete anæsthesia and when that stage is reached turn off the gas and remove the animal from the jar. Note the time it takes for the animal to recover.

This experiment may be varied by connecting a tank of oxygen to the inlet tube in addition to the nitrous oxide and admitting small percentages of oxygen to the bottle at the same time as the nitrous oxide is passing in. Note the difference in symptoms from those seen when nitrous oxide alone was given.

Ethyl Chloride.

The action of ethyl chloride in producing general anæsthesia may be demonstrated as follows:—

Place a white mouse in a small beaker which has a little absorbent cotton in the bottom of it, and then spray a few

drops of ethyl chloride (Kelene) upon the cotton. Note the symptoms shown by the animal and the length of time it takes to produce full anæsthesia. Immediately this stage is reached remove the animal and observe the time it takes to recover.

Magnesium Sulphate Anæsthesia.

Depression of the central nervous system and general anæsthesia may be produced in rabbits by the subcutaneous or intramuscular injection of magnesium sulphate in doses of 1.7 G. per kilo of body weight. This dose can be considerably lessened if sodium oxalate is administered at the same time as the magnesium is given. The anæsthesia and depression may be entirely recovered from if a calcium salt be given later as calcium antagonizes the magnesium action.

Experiment:—

Inject intramuscularly into a rabbit 1.2 G. magnesium sulphate per Kg. body weight and 0.2 G. sodium oxalate per Kg. In from 1½ to 2 hours the animal should be in a state of complete anæsthesia. Now inject slowly either into an ear vein or into a jugular 6 or 8 cc. of a 3% solution of calcium chloride. Note the immediate effect upon the respiration and upon the general condition of the animal.

Local Anæsthesia.

Ethyl chloride may also be used to produce local anæsthesia. This action is explained by the great volatility of the substance so that when it is sprayed upon the skin it evaporates very rapidly causing extreme chilling and finally freezing of the part.

Holding a tube of ethyl chloride in one hand direct a fine spray of the drug against the back of the other hand.

The tube will have to be held at a distance of 18 inches or 2 feet from the object sprayed. In a very few moments the area of skin will become white and examination of its sensitiveness by means of a pin will show complete anæsthesia as compared with the surrounding normal area.

The action of other local anæsthetics was illustrated with cocain. The action of this drug and of its allies, novocaine, etc., is entirely different from ethyl chloride as it is due to a paralysis of the nerve endings conveying the sensations of pain and touch.

The Digitalis Series.

This series embraces a large number of drugs which resemble each other in their pharmacological action. While they act on various organs in the body, the cardiac action is characteristic of the group and distinguishes it from all others. The members of the series are derived from plants widely distributed in nature and as widely separated in their botanical relationships.

The most important is digitalis, which is obtained from *Digitalis purpurea* (purple fox glove); *strophanthus* from *Strophanthus hispidus*; and squills from *Scilla maritima*.

I. Prepare the tincture of digitalis according to the U. S. P., using 25 G. of the powdered drug and the proportionate amount of 75% alcohol. (Made by adding one volume of water to three of alcohol.)

Define a tincture.

This tincture is to be used in the following experiments:

II. To 5 cc. of the tincture add 15 cc. of distilled water. Is a precipitate formed? Cork and set aside the mixture and examine it after a few days.

III. Examine the tincture for the presence of tannic acid by adding ferric chloride.

IV. To 5 cc. of the tincture add two drops of mercury-potassium iodide. Is a precipitate formed? What does the result indicate?

V. Evaporate over a water bath 10 cc. of the tincture to about one-half its volume and then make up the concentrated tincture to the original bulk by adding physiological salt solution.

Now, pith a frog and tie it on a board. Expose the heart and inject into a lymph sac 1 cc. of the modified tincture. Record all changes in the heart.

Describe any change in the rate of the organ and also any differences in the size of the various chambers or in their manner of contracting or dilating.

VI. Biological assay of tincture of digitalis.

Digitalis preparations vary considerably in their strength and it is impossible to standardize them by any chemical means such as can be employed in the cases of those drugs whose activity depends upon alkaloids which may be isolated in pure form and weighed.

It is very hard to isolate the glucosides in pure form as they are likely to break up, making the results obtained very unreliable. For this reason a pharmacological or biological estimation of the strength of a preparation is employed as follows:—

Evaporate 10 cc.¹ of the tincture over a water bath to about half volume² and then make it up to the original volume by the addition of physiological salt solution. Select 3 frogs of about the same size, weigh them carefully (within 0.5 G.) and copy the markings on the back of each animal so as to be able to identify them later. Into the anterior lymph sacs of the frogs inject by means of a glass pipette doses of the modified tincture as follows:—

The doses to be given have to be calculated according to the weight of the frogs used. Frog No. 1 is to receive

¹ All measurements must be made with the same attention to accuracy as in quantitative chemical analyses.

² Why?

0.004 cc.¹ multiplied by the number of grams body weight of the animal; No. 2 receives 0.0055 cc. per gram body weight, and No. 3 0.007 cc. per gram body weight. (These small quantities may be most accurately measured by diluting the modified tincture still more with salt solution and then injecting the proportionately larger dose. The amount of fluid given each animal should measure about 0.5 cc.)

Keep a record of the time when each injection is made. Replace the frogs under the bell jar and at the end of one hour from the time of each injection examine the condition of each frog's heart. Pith the animal if necessary, tie it on the frog board and expose the heart according to the usual method. To have a correct "end reaction" the heart should have just ceased beating with the ventricle in systole and the two auricles markedly distended with blood. Light mechanical stimulation of the ventricle may call out a local contraction but there should be no general beat of the ventricle. (Compare Exp. V.) If all three hearts are still beating when they are examined the doses were too small and must be increased in fresh frogs of corresponding weight. If, on the other hand, the frogs have been dead for some time, the doses were too large and must be made smaller in other frogs.

Having ascertained now by this method the toxic strength of a tincture it may be adjusted by dilution or concentration so that its strength may correspond to any standard which may have been adopted.

The method of assay described is of considerable importance as it or some of its modifications is employed by

¹ Three sections of the class may assay the same tincture by varying the doses to be administered by 0.0005 cc. so as to fill in the gaps in the figures given above. In this way a complete assay may be obtained.

pharmaceutical firms for the standardization of the cardiac remedies of the group (see page 272).

The remainder of the tincture is to be saved to be used in subsequent experiments on blood pressure, etc.

The effects of digitalis on the mammalian heart, both when normal and also when in a state of auricular fibrillation and on the arterial blood pressure are studied later. (Exps., pages 207 and 200.) Its effect on the excretion of urine (diuretic action) may be studied in the rabbit (note, page 239), and the vascular changes in the intestinal and renal vessels according to directions given on pages 299 and 304.

Infusion of Digitalis.

Another preparation of digitalis which is largely used in some parts of the world in place of the tincture and which it resembles in most of its actions is the *Infusion*.

I. Prepare the infusion of digitalis according to the U. S. P.

Define an infusion.

II. Test the infusion for glucosides with tannic acid.

III. Fill a test tube about half full with the infusion, place the thumb over the mouth of the tube and shake it up thoroughly. Note the soap-like character of the foam. The infusion contains a glucoside, digitsaponin, which possesses the property of forming a frothy solution and of holding insoluble bodies in suspension.

IV. Pith a frog, tie it on the board and expose the heart as usual. Inject into a lymph sac 0.5 cc. of the infusion. Compare the effects with those noticed in the experiments in which the tincture was employed.

V. The infusion of digitalis may be assayed by the method described under "Tincture" (Exp. VII, page 171), employing, of course, correspondingly larger doses of the infusion. Also, in making the assay, it will not be necessary to evaporate the preparation as was done with the tincture. Why?

VI. Save the remainder of the infusion and examine it from time to time and note any changes taking place in it.

Examine digitalis leaves (*Digitalis purpuræ*);
strophanthus seeds (*Strophanthus hispidus*);
and squills, the sliced bulb (*Scilla maritima*).

Also examine the following preparations:—

Tincture of strophanthus;

Tincture of squills;

Syrup of squills.

Therapeutic Uses.

Digitalis and its allies are employed principally in certain affections of the heart. When a valve of the heart is diseased so that it does not perform its functions properly, the heart has an increased amount of work to do. In most cases its walls increase in thickness (hypertrophy) to allow of the extra work being done, in other words the heart becomes "compensated." But if an unusual demand is made on the heart before it has time to hypertrophy, the walls stretch on account of their weakness, and the heart is said to become "dilated." When this occurs the contractions are weak and imperfect and the result is that the circulation becomes slowed and the blood tends to collect on the venous side of the vascular system, resulting in a passive congestion of the organs, and in marked cases a generalized œdema of the body may follow.

In such conditions digitalis is of the greatest value. As was seen in its effect on the frog's heart, it increases the strength of the contractions and in some cases lessens the extent of dilatation. The heart begins to pump more blood into the arteries, thus improving the nutrition of the body cells. One of the first organs to be benefited is the heart itself through its coronary arteries. From its improved blood supply it becomes stronger and may be able to hypertrophy and thus carry on its increased work without the further use of digitalis. The improved circulation re-

sults in a disappearance of the congestion of the organs and of the œdema.

The removal of the œdema is also aided by the diuretic action of the drugs belonging to this series; this appears to be due mainly to the cardiac action (cardiac diuretic).

The use of the drug in conditions of auricular fibrillation is demonstrated in a later experiment.

Aconite.

Several alkaloids are derived from plants belonging to the *Aconitum* genus, and among them *Aconitine* is the most important. It is obtained from the root of the monk's hood (*Aconitum Napellus*).

The most important preparation containing aconitine is the tincture of aconite, the alkaloids being very rarely used as different preparations vary considerably in activity, owing to the fact that aconitine decomposes with great readiness.

I. To 2 cc. of the tincture of aconite add an equal amount of water. Try to dissolve the precipitate formed by adding some 95% alcohol. The precipitate in this case consists of resinous bodies (extractives) which are thrown out of solution by the water but which are redissolved by the alcohol.

II. Add 10 drops of tincture of aconite to about 1 cc. of distilled water in a clean beaker. Take the solution in the mouth and retain it there without swallowing for about a minute, after which it may be ejected and the mouth rinsed with water. Observe whether any unusual sensation comes on in from five to ten minutes in that part of the mouth with which the aconite solution has come in contact.

These symptoms, which are due to stimulation of the sensory nerve endings, are characteristic of two drugs, aconite and veratrine.

III. Pith a frog, tie it on the board and expose the heart by the usual method (page 14). Inject into a lymph sac 0.5 cc. of tincture of aconite and keep a record of all changes taking place in the organ, noting not alone changes in rate, but also in the character of the contractions.

After some minutes (10) the heart may become very irregular, this irregularity showing especially in the ventricle. If this change in the rhythm does not develop in a reasonable time it may be necessary to give a second injection of the aconite. The observation of this phenomenon should not be omitted. Keep the heart moist with salt solution.

IV. Biological assay of aconite.

The class may carry out a biological assay of the tincture of aconite according to the method described in the Pharmacopœia. Three guinea pigs may be injected after they have been carefully weighed. The dose given the first shall be the standard dose demanded in the Pharmacopœia, viz.: 0.0004 cc. per gram body weight of the animal; for the second 0.0002 cc. per gram; and for the third 0.0006 cc. per gram. These doses will give a rough estimate of the strength of the tincture, and this estimate may be still further narrowed down by subsequent injections into fresh animals employing doses determined by the results obtained in the first series of animals.

Examine the root of *Aconitum Napellus*.

Therapeutics.

Aconite is occasionally used in cases of neuralgia on account of its action on sensory nerve endings.

It has been used more or less extensively in the past to slow the heart and to lower the temperature in cases of fever. It is doubtful whether it accomplished either of the effects when given in therapeutic doses and its use has therefore been largely given up in favor of the newer and stronger drugs of the antipyrine group.

The Effect of Drugs on the Frog's Heart.

Pith a frog, tie it on a board and expose the heart as usual. Dissect out the vagus nerve and stimulate it with a weak tetanizing current from the induction coil. To find the vagus nerve in the frog: expose the heart, split the pectoral girdle entirely and draw the anterior limbs well apart. Carefully remove the skin and connective tissue in the neck and two large nerves (9" and 12") will be seen on either side passing forward. Trace these backward and they will be found to take a turn outward to enter the base of the skull. The vagus in this position lies between them, running parallel to them in this part of their course. The electrical stimulation of the nerve should be with a weak current and not unduly prolonged as the nerve is fatigued very easily.

Describe the changes in the heart caused by vagus stimulation.

NOTE.—In a certain number of cases the vagus in the frog is not active and it will therefore be impossible to affect the heart by its electrical stimulation. See that the heart is kept moist with salt solution and that it is not injured by instruments or rough handling as it is very easily damaged.

In this frog or in a second one if necessary, examine the action of chloroform by putting the animal, with the heart exposed, under the bell jar in which there is a piece of absorbent cotton soaked in chloroform.

Record all changes seen in the heart, whether changes in rate or in the condition of the various chambers.

If the effects become very marked, remove the animal from the bell jar and allow the heart to recover. Keep it moist by dropping on it a small amount of physiological salt solution.

When the organ has recovered repeat the experiment, using ether instead of chloroform.

Compare the actions of the two drugs. (See Anæsthetics, page 160). Also compare the action of chloral on the heart. (Page 92.)

After the heart has returned once more to normal, put a drop of a pilocarpine (or muscarine) solution on it and note any change in rate. After a few minutes add a few more drops of the same solution and note any further change. Compare the appearance of the heart with its condition under vagus stimulation. If the heart should stop contracting, put two drops of an atropine solution on the organ and if necessary, after a few minutes make a second or third application of atropine.

If the heart begins to beat once more apply pilocarpine solution again and see if it will stop it. Also try stimulation of the vagus nerve. If pilocarpine will not stop the heart, try chloroform by exposing the organ to the fumes as before.

From your knowledge of the anatomy and physiology of the vagus nerve try to account for the effects induced by the drugs as seen above, considering them in the following order, pilocarpine, atropine, pilocarpine and chloroform.

The effects of digitalis, aconite, chloral and paraldehyde on the heart are studied elsewhere. (Pages 171, 183, 92.)

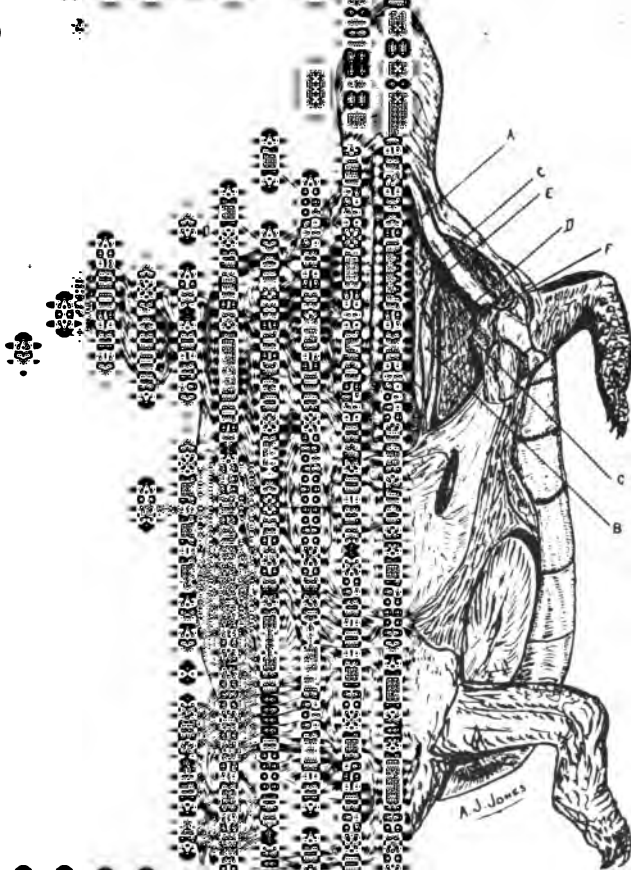
The Effect of Drugs on the Turtle's Heart.

Destroy the brain of a turtle by a blow with a hammer, and turning the animal on its back, tie it on an operating board. Draw the head out so as to put the neck on the stretch and fasten it in this position by means of a nail driven through it. Remove the lower shell (plastron), cutting the lateral attachments with bone forceps or a saw; after cutting the hard parts the shell can be raised slightly and the underlying soft parts severed with a scalpel, taking care to cut toward the shell. Carefully cut away the skin and fascia near the base of the neck, and if the coracoids (G) and clavicles (F) are in the way they may be cut back with bone forceps and a scalpel. Large white muscles (B. B.), the long retractors of the head (*Retrahens capitis collique*) will now be exposed, running back from the head and placed deeply in the neck on either side of the median line. Two other large white muscles (*coraco-hyoideus*, A) will also be seen passing on either side from the front legs forward toward the median line of the neck. Two nerves (C, D) may be found emerging from under the long retractors just posterior to the point where the *coraco-hyoidei* unite in the median line. In the upper part of their course they lie internal to the long retractors, then wind around the muscle to reach its upper surface (in this position of the animal) and pass down the neck, lying on the muscle. One of these nerves is the vagus (D), which must be identified by gently separating it from the surrounding structures and stimulating it with the electric current, watching the effect on the heart. When it is found, place a ligature around it. In dissecting out the nerve be careful not to take hold of it

GUIDE IN

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the pericardium. Thread
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Vagus, E. Carotid artery,
D, cut end, H. Heart.

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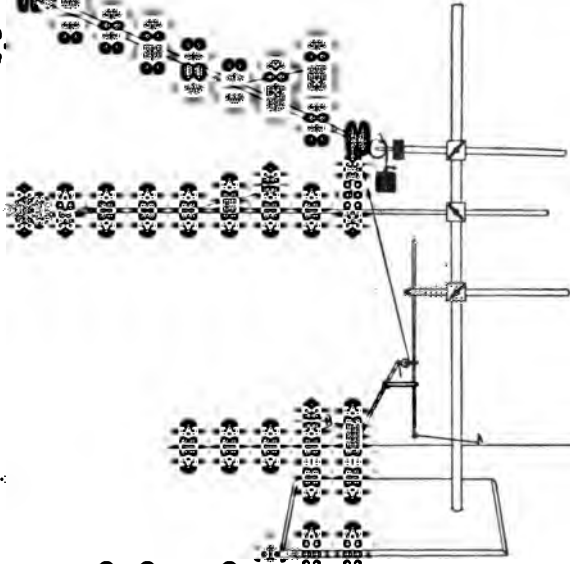


Fig. 1. Myograph stand for the turtle's heart.

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ventricle. Vary its height so that it will record the movements of the heart on the smoked surface of a drum properly placed. Arrange the stationary lever (D) to draw a base line just below the tracing of the cardiac movements.

After taking a short tracing showing the normal contractions of the heart, take one showing the effects of stimulation of the vagus.

NOTE.—Mark on the tracing when any stimulation is begun or stopped or when any drug is applied.

After the heart has recovered and while the record is continuing apply slowly 8 or 10 drops of a 0.5% nicotine solution near the base of the heart where the large vessels enter. This should be sufficient to cause distinct slowing of the heart.

After the heart has recovered from the nicotine try the effect of vagus stimulation again.

Continuing the tracings, apply a few drops of a pilocarpine solution (0.5%) to the heart. When the contractions become very infrequent (and not until then) or stop entirely, apply atropine (0.1%) to the heart, adding a drop or two at a time to the organ.

When the contractions return to normal, stimulate the vagus with the electric current as before.

Apply pilocarpine again to the heart. Compare the tracings with those obtained earlier in the experiment.

Shake 1 cc. of chloroform in 20 or 30 cc. of physiological salt solution and pour some of this solution from time to time over the heart. When the cardiac contractions become very weak, remove the chloroform solution with a piece of absorbent cotton and assist the recovery of the heart by bathing it in fresh salt solution.

Note that pilocarpine does not stop the heart after atropine, nor has vagus stimulation any effect. Chloroform, on the other hand, acts after atropine. How is this to be explained? How did nicotine produce its effect?

Remove carefully the paper with the tracings from the drum and fix it by passing it through a solution of shellac in alcohol.

Study the tracings according to directions given on page 215.

The Effect of Drugs on Blood Pressure.

Anæsthetize a dog, cat or rabbit according to the methods described earlier (page 11), and when the animal is completely under the influence of the anæsthetic, tie it on its back on the operating board and remove the hair from the neck region. Make an incision about four inches long in the median line of the neck and expose the trachea; insert a tube (page 18) to allow of artificial respiration should it become necessary.

Expose the jugular vein on one side and insert a cannula (page 16). On the opposite side expose the carotid artery and in like manner tie a cannula in it (page 17).

Fill the venous cannula with physiological salt solution and the carotid cannula with a solution made by mixing equal volumes of water and a saturated solution of sodium sulphate. (See note 2, page 17.)

The ordinary form of mercury manometer, which is so well known as to need no description, is used. Fill the proximal limb of the U-shaped tube, i. e., the limb which is to be connected with the blood vessel, with the sodium sulphate solution, using for this purpose a pipette with a long tapering point. The rubber tube, which is connected to the side tube of the proximal limb of the manometer, is to be completely filled with the sulphate solution, taking great care to see that all air is expelled from the tube and manometer. Close the clamp on the tubing which connects the rubber tube and manometer. With a short piece of rubber tubing connect a large pipette (25 cc.) filled with the sulphate solution to the upper opening of the proximal limb of the manometer and blow in the pipette so as to raise the

pressure until there is a difference of 10 or 12 cm. in the height of the mercury in the two limbs.¹ Retain this pressure by closing the piece of rubber tubing with a spring or screw clamp and disconnect the pipette.

Connect the distal end of the rubber tube which is attached to the side tube of the manometer to the carotid cannula and open the clamps so as to allow the carotid pulsations to be transmitted to the mercury. Arrange the writing lever so that it will record the pulsations on a blackened drum with a time marker writing below the tracing. Keep the writing lever in light contact with the drum by means of a small weight suspended by a thread from a support above the drum, taking care that the thread does not press too hard against the lever.

Administer the following drugs in the manner described, taking a short piece of normal tracing before each is given, and waiting until the effects of one drug have passed off before applying the next. Observe the precautions as to the injection of any air.

1. Amyl nitrite: 3 drops on absorbent cotton applied to the animal's nose or to the tracheal tube if that has been inserted.

2. Chloroform: given on absorbent cotton as above.

3. Ether: given on absorbent cotton.

4. Suprarenal extract. Adrenalin chloride (1-1000); 1 drop in salt solution injected into the vein. Wash the cannula and syringe thoroughly before injecting the next drug.

5. Nicotine chloride 1 mg. diluted with salt solution.

¹ The pressure may also be raised by means of a pressure bottle partially filled with sulphate solution and suspended from the ceiling. A tube from this bottle may be connected with the manometer in the manner described above.

6. Pituitary gland extract (Pituitrin 0.5 cc.) in salt solution.

7. Digitalis. 1 cc. of the tincture from which the alcohol has been evaporated (given intravenously).

8. Barium chloride 20 mg. in salt solution.

Allow the drum to continue rotating a short distance after the heart has stopped, so that the writing lever traces a straight line from which measurements may be made to ascertain the absolute blood pressure. Remove the tracings from the drums and fix them by passing them through a solution of shellac in alcohol.

The blood pressure tracings are to be analyzed according to the directions given on page 215.

Effect of Drugs on the Mammalian Heart and Blood Pressure.

Anæsthetize a dog (page 12) and when anæsthesia is complete, tie the animal on its back on the operating table. Make an incision through the skin in the median line of the neck, and after exposing the trachea insert a cannula in the usual way (page 18). Expose a vein, either one of the jugulars or a saphenous in the leg, and insert a venous cannula.

Also dissect out an artery, either one of the carotids or a femoral and insert a cannula into it and arrange it for blood pressure tracing according to the method described in the previous experiment.

Dissect out the vagus nerve on one side, tie a ligature around it and cut the nerve between the ligature and the head.

Continue the skin incision in the median line from the neck down the thorax to an inch or two below the end of the sternum, and deepen the cut to the bone. Stop all bleeding points with artery forceps.

Connect the tubing of the bellows with the tracheal tube and start artificial respiration.

Saw through the entire length of the sternum, keeping exactly in the median line and complete the division with bone forceps; draw the two sides of the thorax well apart and secure them to the sides of the table with sharp hooks.

Regulate the amount of air entering the lungs by means of a screw clamp placed on a piece of rubber tubing on the arm of the tracheal cannula.

NOTE.—During the process of sawing through the sternum and opening the thorax the animal must be very deeply under the anæsthetic, otherwise the heart may go into delirium.

Cut both phrenic nerves as they pass down to the diaphragm lying in plain sight on either side of the pericardial sac.

Open the pericardium along its entire length with a pair of scissors and fasten it to the cut edges of the sternum by means of four or five stitches.

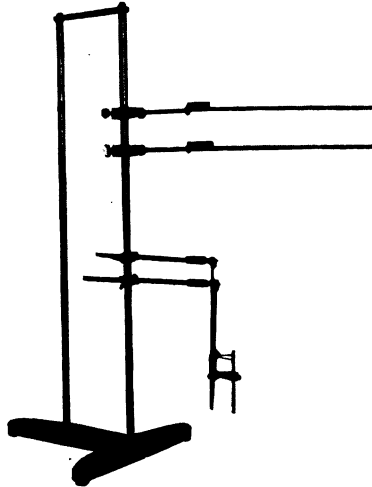


FIG. No. 8. Myocardiograph for dog's heart.

Take a stitch in either side of the right ventricle with small curved needles and thread, and tie the thread into the heart in the same way as you did with the turtle. Similarly take stitches in the right auricle. If bleeding should occur from the needle passing completely through the wall of either chamber take up the bleeding point with a pair of dissecting forceps and tie a ligature around it.

Attach the two sets of levers of the dog's myocardiograph¹

¹ The dog myocardiograph (Fig. 8) is constructed on exactly the same principles as is the myocardiograph for the turtle's heart (Fig. 7), excepting that, as is shown in the figure, it is double, so that it will record the movements of both auricle and ventricle. The apparatus has other modifications as is seen in the plate, but the principle is not essentially altered. A description of that part of the apparatus which is connected to the heart is found in the *Jour. of Physiol.*, 1897, XXI, p. 213.

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er, Fig. 9); continue

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intravenously.

(curarin) 0.5 cc. in salt

7. Amyl Nitrite. 3-5 minims dropped into the side of the tracheal cannula.

8. Nicotine chloride. 2 mg. in salt solution.

9. Digitalis. 2 cc. of the tincture which has been evaporated to one-half volume and made up to original bulk with salt solution.

10. Digitalis. Toxic doses. Give 3 cc. doses repeatedly until the animal dies allowing about 4-minute intervals between injections.

Mark the tracings carefully so that they can all be identified and fix them in shellac as before. Analyze them according to directions given on page 219.

Cut off the head of the animal and as soon as convenient dissect out the submaxillary ducts according to the directions given on page 255. This dissection will allow more speedy operating on the living animal in the experiment on the salivary secretion.

Analysis of Tracings.

Graphic records have been obtained in three experiments (turtle's heart, dog's heart and blood pressure) and it is of first importance that these records should be carefully analyzed in order to properly study the changes induced by the various drugs. A few hints are given below to call attention to some of the more important points to be looked for, and some suggestions are made as to the best way of studying the tracings.

Tracings Obtained from the Turtle's Heart.

It is rarely necessary in these records to take accurate measurements, as the changes induced by the drugs are generally quite marked.

Describe the effect of stimulation of the vagus, noting whether the heart stops in systole or diastole. Likewise, describe the condition of the heart under pilocarpine (before and after atropine administration) and also the effect of the atropine.

Under chloroform note especially the strength of the heart as indicated by the extent of contraction and of dilatation, which is measured by the distance of the tracing from the base line.

Summarize the actions of the different drugs.

Tracings from the Blood Pressure Experiment.

Study these first with regard to changes in pressure. Take careful measurements of the distance of the tracing from the base line at various points along the course of the curve;

viz., before the drug is injected to get the normal; after the action of the drug becomes apparent; and later at short intervals of time which will depend upon the drug used. Where the curve is fairly uniform readings every thirty seconds are frequent enough.

Measuring in this way from the base line will only give relative changes in pressure, which is all that is necessary in these experiments. To get the absolute pressure, the zero line which was marked on the tracing would have to be used, measuring its distance above or below the base line and adding or subtracting this distance from the figures obtained above. This result must then be doubled as there are two arms of mercury in the manometer.

The changes in the rate of the heart are studied in the following manner: Draw two lines parallel to each other and perpendicular to the base line so that they shall intersect the tracing. The distance of the lines from each other is determined by the rate of the drum as indicated by the time marker; they are usually drawn to include between them a space equal to five or ten seconds. Count the heart beats in the tracing included between the two lines and estimate the rate of the heart per minute. These estimations are made along the curve at the same points as the pressure changes are measured.

Also describe any other changes in the curves, such as irregularities of the heart or of the respiration, etc.

Summarize the effects of the different drugs upon the pressure of the blood and upon the rate of the heart.

Tracing from the Dog's Heart.

These are to be studied and analyzed by a combination of the methods outlined above. The rate of the heart is ascertained as described under the directions for studying the blood pressure tracings, while the strength of the organ as indicated by the extent of systole and diastole is measured from the base line. Describe all other changes in the tracings, and in your notes make a full summary of the actions of the different drugs.

Digitalis in Auricular Fibrillation.

One of the most important disturbances of the heart in which digitalis is employed and in which it probably exerts its most favorable action is in Auricular Fibrillation. In this condition there is no orderly contraction in the auricle, but due perhaps to excessive irritability of the muscle there is a constant fibrillary twitching in all parts of the auricular wall. These fibrillary contractions not only prevent the auricle from emptying itself properly, but also incessantly bombard the auricular-ventricular bundle. These stimuli coming to the bundle so rapidly, only a certain number of them can get through and some of these, coming at irregular intervals, will strike the ventricle when it is in its refractory stage. The net result of these irregular stimuli upon the ventricle is to make it very irregular with consequent marked arrhythmia of the pulse. At first these attacks of auricular fibrillation may be quite transient and occur at long intervals, but later on they may be more frequent and gradually the condition becomes permanent.

In this form of cardiac disturbance, as stated, digitalis is of great value and it will frequently restore the ventricular rhythm to a state of almost complete regularity. This may be accomplished either by an effect upon the ventricle itself, altering it in such a way that it does not respond so readily to the rapid and irregular stimuli coming down from the auricle or it may interfere with the passage of the stimuli through the Bundle of His so that fewer reach the ventricle. In any case the net result of the administration of digitalis is a disappearance of the cardiac arrhythmia and an improvement in the circulation. There is no change in the

fibrillary contractions of the auricle only their influence on the ventricle is removed.

This condition of Auricular Fibrillation can be produced experimentally and the action of digitalis upon it demonstrated.

The early part of the experiment is carried out and the dog prepared in the same manner as was outlined in the work upon the dog's heart (page 207). After the carotid has been prepared for a pulse tracing or blood pressure and the myocardiograph has been attached to the heart, the two wires from a secondary coil are attached to the two poles of that portion of the myocardiograph which is attached to the auricle, and a weak tetanizing current is passed through the auricle. The chamber responds immediately by passing into a condition of fibrillation. The electric current must not be too strong or it may spread to the ventricle and set up delirium in that chamber also. When a satisfactory strength of current is found, the auricular recording lever should show merely an irregular twitching while that from the ventricle should record a strong but very irregular contraction. The pulse tracing would also naturally be irregular.

The tincture of digitalis (from which the alcohol has been evaporated and its original volume made up by salt solution) may now be injected intravenously in 3 cc. doses, the injections not being repeated at shorter intervals than about four minutes. After a few of these injections have been given in the manner described, the heart will show the effect of the digitalis in a strong regular beat of the ventricle. regular pulse, and probable increase in blood pressure. The auricle will still show its fibrillary contractions.

The importance of the action of digitalis in this condition is shown by the fact that it is said that of all forms of cardiac arrhythmias auricular fibrillation constitutes about 40%.

Perfusion of Blood Vessels.

The experiment can be carried out either upon the kidney or other organ such as a spleen removed from a dog, cat or rabbit or upon the vessels of a frog. The latter animal proves very successful and that method will therefore be described first. Select a fairly large frog (30 G.-40 G.), pith it and expose the heart and insert a cannula in one aorta, pointing it away from the heart including the second aorta in the ligature with which the cannula is tied. Make a small cut in the sinus venosum so that the fluid which is perfused through the vessels can escape. Suspend the frog by its jaw and place under it a receptacle in which the outflow can be caught. About a foot above the frog place a small pressure bottle (500 cc.), the lower opening of which is fitted with a piece of rubber tubing upon which is a snap clamp. Fill the aortic cannula with Locke's solution or physiological salt and fill the pressure bottle about half full of the same solution. When all the air is excluded from the rubber tubing connect it with the aortic cannula. Open the snap clamp which has been placed on the rubber tube and allow the perfusion to proceed, measuring the outflow every five minutes. After about three readings there should be a fairly uniform outflow and 200 mg. of sodium nitrite are added to the fluid in the bottle and the solution mixed thoroughly. The readings of the outflow are continued and after one or two the amount obtained should be very considerably increased. If it is not increased within a reasonable time further nitrite may be added. Now replace the nitrite solution in the flask with fresh salt solution and wash out the frog's vessels, seeing whether the

outflow will return to normal. After two or three readings add to the solution in the flask 1 cc. of a 1-1000 solution of adrenalin. Continue the readings until a marked decrease in outflow is obtained. In place of the adrenalin tincture of digitalis in the strength of 3 cc. of tincture to 100 cc. of salt solution may be used.

If a kidney or other organ is to be used in place of the frog's vessels the experiment is carried out in essentially the same manner.

The kidneys which have been removed from a dog, cat or rabbit should not be kept any longer than is absolutely necessary after being removed from the animal, certainly not more than twenty-four hours, as after that time post-mortem changes have advanced so far as to interfere with the action of drugs on the vessel walls.

In preparing the kidneys for the experiment it is usually best not to remove the capsule, as in so doing the parenchyma is frequently torn. Remove the fat and connective tissue from around the vessels and insert a glass cannula in the artery. Fill the artery and cannula with salt solution. Fasten a small glass reservoir (say 250 cc.) about 1.5 meters above the table and connect a long piece of rubber tubing with its lower orifice, and fill reservoir and tube with Locke's solution and clamp the tube.

Connect the lower end of the rubber tube with the arterial cannula taking care that all air is expelled. Remove the clamp from the tubing and place a receptacle under the kidney to catch the fluid after it has passed through the vessels and continue the experiment as described above for occasions where a frog is used.

What do the changes in the rate of outflow under the two drugs indicate in regard to their action on the blood vessels? How do the results help to explain the observations made in the experiment on blood pressure (page 200).

Action of Drugs on Isolated Tissues.

The action of many drugs in the body may be studied to advantage by observing the effects of these drugs upon isolated tissues or organs, which during the time that they are in use are under as nearly normal conditions as possible as far as temperature and oxygen supply are concerned. Those structures which are most commonly employed in this work are the uterus, or strips of intestine or blood vessels.

While more or less complicated apparatus is needed if quantitative results are desired, yet if the requirements are only qualitative quite a simple arrangement is all that is demanded. The Harvard muscle warmer and heart lever, kymograph, oxygen or air supply, and warm Locke's solution will answer all purposes.

In case the uterus is to be used, a guinea pig (or rabbit or cat) weighing 200 G. or 300 G. is killed by a blow on the head and the abdomen opened and uterus exposed and a fine thread tied around the ovarian end of one horn. After the organ is freed from the posterior wall, another thread is tied around it near its lower end, and it is then removed from the body. Using the thread on the vaginal end of the organ, the uterus is now tied to the end of the bent metal rod of the muscle warmer. The thread on the ovarian end is passed through the round opening in the plate disc and fastened to a muscle lever so that the lever will record the contractions of the uterus upon a lightly smoked drum. Uteri differ considerably in activity as some are so active that it is necessary to weight the lever in order to diminish the extent of the contractions while other organs are quies-

cent unless aroused to action by some drug. The lower end of the glass tube of the muscle lever is fitted with a piece of rubber tubing which can be closed by a snap clamp, and the tube is then adjusted in place around the piece of uterus, and filled with Locke's solution at a temperature of 38° C. Air or oxygen is led to the bottom of this tube by means of a capillary glass tube or a fine rubber catheter. For long experiments or for any exact work the muscle warmer should be enclosed in an outer bath of water maintained at body temperature, but for short experiments this is hardly necessary as the Locke's solution is replaced at short intervals, after the use of each drug, by fresh solution of the proper temperature. When the lever is recording properly and the drum is moving at a slow rate, the actions of the following drugs may be studied by adding them in turn to the solution bathing the uterus. When the climax of the action is reached, the used solution should be drawn off and replaced by fresh and the uterus allowed at least five minutes to recover before another drug is added.

Add to the solution bathing the uterus 1 drop of adrenalin (1-1000). When the maximum effect is obtained, draw off the solution and replace it with fresh warm solution as directed above. After giving the organ a rest, add 1 or 2 mg. of pilocarpine and then immediately after putting fresh Locke's solution on the uterus give 1 to 2 mg. Atropine sulphate. This may be followed in turn by pilocarpine again ; Fl. Ext. Ergot 0.3 cc. ; Pituitary Extract (Pituitrin 2 drops) ; each in a fresh solution.

The doses given above may have to be modified to suit the requirements of the individual organ. When the ex-

periment is completed, fix the tracings with shellac and later study them carefully, taking up the action of the individual drugs, comparing the results with those obtained with these drugs in other experiments.

Diuresis.

Anæsthetize a rabbit with paraldehyde (page 11) and when anæsthesia is complete tie the animal on the operating board. Cut the hair from the neck and also from the lower part of the abdomen. Expose a jugular vein and insert a cannula as usual.

To insert a cannula in the bladder to collect the urine, make an incision about an inch and a half long in the median line of the abdomen, beginning the incision at the symphysis and extending it upward the distance named. Deepen the cut until the peritoneal cavity is opened. The bladder is usually found partially distended and lying amid folds of fat. Draw it out through the opening made and empty it by gentle pressure, catching the urine in a dish as it flows from the urethra. Save this urine to be tested for the presence of sugar.

Tie a knot loosely in a piece of thread and lay it on the abdomen so that the knotted thread will encircle the bladder and be ready to tie the cannula in place when the latter shall have been inserted.

After locating the position of the ureters, so as to avoid including them in the ligature, take hold of the upper surface of the bladder on the two sides with two pairs of forceps and draw them gently up and outwards so as to exert a slight tension on the organ. While one operator holds the bladder in this way the other operator makes a cut with a sharp pair of scissors midway between the forceps in the fundus of the bladder. The cut will vary with the size of the cannula flange (a, Fig. 10) and will probably average about two centimeters in length. Into this incision now

place the cannula so that its flange shall be entirely surrounded by bladder tissue and tie it in place with the loose ligature already mentioned. Examine it after it is tied to see that the ureters are not included.

Fill the bladder and cannula with warm physiological salt solution and connect the end of the cannula with the piece of bent tubing (b, Fig. 10) which can now rest on a support

so as to extend over the end of the operating board where small weighed dishes are to be placed to catch the urine.

As soon as the urine begins to drop from the tube a record of the time is to be made, and thereafter to the end of the experiment, every five minutes, the dish with the urine is to be replaced by a clean receptacle and the first one weighed, and the weight of urine recorded.

During the course of the experiment the animal should be kept covered with a towel or other cloth in order to retain as much of its body heat as possible.

Take two or three readings until a fair regularity is obtained, and after this normal is ascertained inject into the vein 40 mg. of theocin¹ dissolved in hot water.

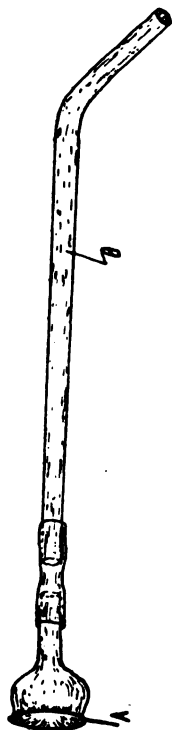


FIG. No. 10. Bladder Cannula. A. Flange to be tied in bladder. B. Bent delivery tube.

¹ Theocin is artificial theophyllin. A like quantity of caffeine might be given in place of the theocin except that the action of the caffeine lasts much longer and may prolong the experiment unduly.

After this injection the urine will probably be increased and the next drug must not be injected until the flow has returned to about the normal.

Test some of the urine which was in the bladder at the beginning of the experiment for sugar by means of the Fehling test. If none is found make one or two tests later in the course of the experiment with urine which is excreted under the action of the theocin or sodium nitrate until sugar shall be found.

The excretion of iodide in the urine may also be studied by the careful injection intravenously of 30 mg. sodium iodide. The urine passed later is tested for iodide as follows: add a few drops of concentrated sulphuric acid to the urine to be tested, and to this some 10% sodium nitrite solution and some starch paste which if iodide is present should be colored blue.

When the amount of urine has fallen, inject 0.250 G. (5 cc. of a 5% solution) of sodium nitrate and collect the urine as before.

NOTE.—If time allows, the diuretic effect of digitalis may be studied in the same way.

Caffeine, sodium nitrate and digitalis are taken as representatives of the large class of drugs known as *Diuretics* from their action in increasing the amount of urine.

They may act either on the heart or on the kidney, and they are known as cardiac or renal diuretics according to the point of action. Digitalis is an example of the former class, as it causes diuresis indirectly through its effects on the circulation. Caffeine probably acts directly on the renal epithelium, while various salts, of which sodium nitrate is an example, induce diuresis by their salt action (saline diuretic) in addition to which there may be an action on the kidney epithelium.

Therapeutic uses of the Diuretics.

See also under Caffeine and Digitalis, Therapeutic uses. (pages 140 and 179).

These drugs or their allies are chiefly employed in diseased conditions in which the amount of urine excreted is below the normal, a condition which may result from disease of the kidneys or of the heart. In conditions of œdema or ascites, there is an abnormal collection of fluid in the tissues; its removal may be aided by the use of the diuretics. In poisoning with an irritant drug which is eliminated by the kidneys, the latter may be protected from the action of the toxic substance by increasing the volume of urine through the administration of these drugs.

Action of Drugs on the Cervical Sympathetic Nerves and on Intestinal Peristalsis.¹

Anæsthetize a rabbit (an albino if possible) with paraldehyde (page 11) and when anæsthesia is complete tie the animal on the operating board and remove the hair from the neck region. Expose the jugular vein on one side and also the trachea and insert cannulas in each. Expose the carotid sheath on the other side, open it, laying bare the vessels and nerves. The largest nerve seen is the vagus and the smallest is the depressor nerve, while the third is the cervical sympathetic trunk, which is to be separated very carefully from all connective tissues and a fine thread passed under it. Make sure you have the correct nerve by gently raising it and stimulating it with a weak tetanizing current, observing meantime the effect on the pupil on the corresponding side; it should dilate during the stimulation. If the correct nerve has been isolated tie the ligature around it as low down in the neck as convenient and cut the nerve trunk below the ligature. Hold the animal's ears up gently against the light so that the vessels may be clearly seen, and observe the difference in their size on the two sides; note also the difference in the temperature of the two ears. The differences in the vessels in the two ears may be best seen by comparing the smaller branches rather than the main arteries. Compare the size of the pupils on the two sides. How would you explain these changes?

Make a longitudinal incision about 10 cm. long in the middle line of the abdomen extending the cut completely

¹ The experiment on intestinal peristalsis may be run as a separate experiment (see page 292) or as directed here in conjunction with the cervical sympathetic experiment.

through the wall. Pulling the two sides of the wall apart insert in the incision a glass evaporating dish measuring about 10 cm. in diameter. Place the dish between the skin and muscle layers so that it will be held in place by the wall and thus serve as a window. It may be necessary to pull the caecum to one side in order to permit of a free view of the small intestine. Observe closely the normal movements of the intestine.

Now raise the trunk of the cut cervical nerve carefully by the ligature and stimulate it with the electric current as before. During this stimulation look especially for any changes in the calibre of the ear vessels on that side; and for any changes in the position of the eyeball, or of the eyelids, or in the size of the pupil. After the changes have been seen and described, inject into the jugular vein 3 mg. of nicotine chloride. Immediately after the injection notice the effect of the drug on the animal's respiration (make sure the animal starts breathing again, otherwise start artificial respiration), and also look for any movement in the animal's whiskers.

Again stimulate the cut cervical sympathetic comparing the results with those obtained at the first stimulation. (Should they be the same as before, it will be necessary to inject more nicotine.) Now using very careful dissection trace the trunk of the sympathetic toward the head until the superior cervical ganglion is reached. It will be recognized as a small whitish enlargement on the trunk of the nerve. When it is found, stimulate the trunk of the sympathetic beyond the ganglion, that is, between the ganglion and the head of the animal. Compare the results of stimulation at this point with those obtained below the ganglion.

NOTE.—In addition to the nerve fibres which run to the eye by way of the cervical sympathetic and which are distributed to the radial muscle fibres of the iris there are nerves which control the circular fibres. These come from the motor oculi to the ciliary ganglia and run from that point to the irises. The size of the pupil can be changed by drugs acting along the course of these fibres also.

Inject into the jugular vein 5 mg. of pilocarpine (1 cc. of 0.5% solution) and observe any change in the pupil and also in intestinal peristalsis.* (Note any effect on the heart rate.) Now inject 1 mg. atropine sulphate (1 cc. of 0.1% solution). What effect has it upon the pupil, peristalsis and heart rate?

Finally inject a small dose of suprarenal extract (adrenalin 1-1000 solution, 1 drop in salt solution) note changes in peristalsis, calibre of vessels and heart rate.

What effect has nicotine upon the sympathetic ganglia?

Cocaine dilates the pupil by stimulating the terminations of the nerves from the superior cervical ganglia which end on the radial fibres.

Physostigmine (Eserine) acts on the same point and in the same manner as pilocarpine, i. e., on the nerve terminations on the circular muscular fibres; atropine acts at the same place, but instead of stimulating the nerve endings, its action is to paralyze them, removing the pilocarpine effects.

Therapeutic Uses.

Physostigmine (eserine) and, less commonly, pilocarpine are used in ophthalmological practice to contract the pupil of the eye in certain diseased conditions, the most important of which is glaucoma. For this purpose a solution of $\frac{1}{4}$ to 1% is dropped in the eye as often as necessary. Physostigmine is also occasionally used to stimulate intestinal peristalsis in cases of atony of the intestine. For this purpose it is given by hypodermic injection.

Atropine is used to dilate the pupil to permit of ophthalmoscopic examinations, and as a therapeutic agent in many ocular diseases. It is also used in cases of colic or griping to overcome the abnormal contractions of the intestine. *Homatropine*, an artificial alkaloid resembling atropine in its action on the eye, is largely used as a substitute for the latter, as its action is not nearly so prolonged.

Cocaine is also largely employed to dilate the pupil to allow of examination of the interior of the eye.

Mydriatics are drugs which dilate the pupil; myotics contract it.

Salivary and Pancreatic Secretion.¹

Anæsthetize a large dog with morphine and chloretone (page 12) and if necessary supplement them by the use of chloroform. When anæsthesia is complete, expose a vein in each leg and insert cannulas. As a matter of precaution, the trachea may also be exposed and a cannula tied in it to permit of artificial respiration should it become necessary.

The submaxillary duct and chorda tympani nerve are to be dissected out according to the following directions. (This dissection should have been previously carried out on the head of a dead animal (from Exp., page 212) so as to allow greater speed in operating on the living dog.)

“Make an incision 3 or 4 inches long through the skin and platysma muscle along the inner border of the lower jaw, beginning about the angle of the mouth and continuing backward towards the angle of the jaw. Ligate doubly and divide any vessels that come in your way. Divide the digastric muscle about its anterior third and clear it from its attachment. The broad, thin mylo-hyoid will now be seen with its motor nerve lying on it. Divide the muscle across its middle and dissect it up carefully. The lingual nerve is seen emerging from under the ramus of the jaw, running transversely towards the middle line and then passing forward parallel to the larger hypoglossal nerve. In its transverse course it crosses the submaxillary and sublingual ducts. The chorda tympani leaves the lingual nerve and runs backward along the duct towards the gland. Put a glass cannula into the duct in the same way as you would

¹ The salivary and pancreatic secretion experiments may be run separately if preferred.

GUIDE IN

back the lingual and put a back as possible, and then the chorda tympani being Connect the cannula with scale is marked.



Chorda Tympani. A. Chorda Tym-
pani, B. Lingual nerve, D. Mylo-hyoid muscle
cut, C. Gastrocnemius muscle cut, G. Ra-
dial nerve emerging from

duct is being carried on, a the cervical sympathetic log the cervical sympathetic and closely connected with separated by careful dis-

ected if desired.

section, as the fibres run in distinct strands. For some distance below the superior cervical ganglion the two nerve trunks are not connected. After the sympathetic is isolated tie a ligature around it and cut the nerve below it.

If the experiment has been properly done, both nerves (viz., the chorda and the sympathetic) can be stimulated by placing them on electrodes, and any secretion from the gland will pass into the horizontal tube, where it can be measured on the scale.

The pancreatic duct is exposed in the following manner. An incision is made through the abdominal wall about 6 or 8 cm. to the right of the median line and starting from the costal border and extending caudalwards for a distance of about 15 to 20 cm. The duodenum is located and pulled out through the incision and the pancreas is found attached to it for some distance. Starting at the point where the tail of the pancreas leaves the intestine and following up toward the pylorus for about 3 cm. the pancreas is separated from its anterior attachment to the intestine by blunt dissection. At about this point the large duct of the pancreas will be found entering the wall of the intestine. It is very short. The surrounding tissue is separated from the duct and a ligature placed around it. A V-shaped cut is made in it and a glass cannula inserted and fastened by means of the ligature. A small calibre tube is now connected to the cannula by means of a piece of rubber tubing and the pancreatic secretion observed either by means of a scale back of the tube or by the drops falling from the end. The intestinal loop is now returned to the abdomen or may be secured outside protected by warm moist towels and the abdominal incision closed as far as possible.

1. Stimulate the chorda tympani with a weak induction current and watch the effect on the secretion.
2. When the effect of chorda stimulation has passed off stimulate the sympathetic trunk (peripheral or head end) and observe the difference in the quantity and quality of the saliva when compared with the "chorda saliva."
3. Inject 5 mg. of nicotine chloride and observe closely the effect upon the salivary secretion. Watch the effect upon the respiration and heart rate. (Be sure the respiration continues after the nicotine has been injected.)
4. Stimulate the chorda again and compare the results with those obtained in 1.
5. Stimulate the sympathetic and compare with 2.
6. If the nerves are still active, repeat the injections of nicotine, giving 20 mg. each time until no further effect is

Preparation of secretin: The secretin which is to be used in the experiment is to be prepared from the intestine of one of the dogs which has been used earlier in the course. Its mode of preparation is as follows: Immediately after the animal has been killed, open the abdomen and remove about 1.5 meters of the upper part of the intestine. Cut this piece open longitudinally under running cold water and after it is cleansed, spread it flat on a piece of glass and scrape off the mucosa by means of a second piece of glass. Put this material in a large mortar with about an equal volume of fine clean sand and from 100 to 300 cc. of 0.4% HCl, the amount depending upon the bulk of sand and mucosa. Grind this mass now thoroughly for ten minutes so as to get the tissue cells well broken up. Transfer the mixture to a large evaporating dish and boil it for a minute or two, keeping it well stirred. Now add a sufficient quantity of a fairly strong solution of NaOH so as to give an alkaline reaction and follow this with sufficient 25% glacial acetic acid to give a distinct acid reaction. Strain the mixture now through muslin, pressing out all the fluid possible. This fluid, which should be rather strongly opalescent, is now partially sterilized by placing the flask containing it over a water bath for ten minutes and then plugging it with absorbent cotton. Keep it in a cool place until it is needed. It may be injected in this strength or still better diluted with salt solution. The normal pouring out of secretin into the blood may perhaps be best simulated by diluting the stock solution of secretin with many times its volume of physiological salt solution and then placing this solution in a burette and connecting it with a femoral vein, allowing it to enter the blood stream slowly, and continuously. Marked effects on the blood pressure are thus avoided and a constant secretion from the pancreas obtained. This latter method is the one to be used in this experiment.

obtained on nerve stimulation. How do the effects of subsequent injections compare with the primary injections?

7. Trace the trunk of the sympathetic to the superior cervical ganglion and stimulate the nerve beyond, observing if secretion can be obtained.

Inject the following drugs in the order named, observing their effects upon both the salivary and pancreatic secretion:

8. Pilocarpine, 5 mg.
9. Atropine, 1 mg.
10. Pilocarpine, 5 mg., compare with 8.
11. Suprarenal extract, Adrenalin chloride, 1-1000 sol., 5 drops in salt solution.

What effect has nicotine upon the sympathetic ganglia? Compare also the experiment on the sympathetic ganglia in the rabbit (page 247).

As was shown in this experiment, the salivary gland is innervated from two sources, both of which are acted on by nicotine. Pilocarpine and atropine affect the salivary secretion by acting on the cranial nerve supply of the gland. The action of the suprarenal gland extract in causing secretion is believed to be upon the terminations of the sympathetic fibres in the gland cells. (It is not always possible to get a secretion of saliva by the injection of suprarenal extract.)

12. After the effects of the suprarenal extract have passed off, start the infusion of the secretin, giving it quite slowly. In a minute or two the pancreas should begin to secrete actively. When the secretion is well established and while the secretin is still entering the vein inject into the opposite femoral vein suprarenal extract as was done in 11. What effect has this upon the pancreatic secretion and what

explanation can you suggest for it, knowing the effect of the drug upon the blood vessels?

13. Try the effect of an injection of 10 mg. of nicotine. The influence of this injection may not be very marked on account of the earlier use of nicotine in the experiment.

14. Give an injection of pituitary extract (0.5-1 cc. pituitrin). These last two drugs also cause marked constriction of the abdominal vessels.

The action of the hormone, secretin, upon the pancreas is in marked contrast to that of pilocarpine which is so active in producing a secretion from the salivary gland, and it illustrates probably the relative importance of chemical and nervous control over the secretion of this gland. Any drug which constricts the vessels of the gland and thus cuts off its blood supply will in this way inhibit its activity.

Antipyretics.

The temperature of the animal body is determined by the relation between heat formation and heat dissipation. To maintain a uniform temperature in warm blood animals, it is necessary, therefore, to have some mechanism to preserve a balance between these two activities. This heat regulating mechanism has not been shown to be an anatomical or physiological unit or definite center, but on the other hand some of the well-known centers are closely connected with heat control.

Temperature changes in the warm blooded animal are usually brought about by a disturbance of the regulating mechanism. For instance, the temperature may be lowered by depressing the central nervous system as by ether, chloroform or chloral, under the influence of which less heat is formed; or the temperature may be lowered by increasing heat output either by dilating the peripheral vessels and exposing more blood to the outside air or by increasing the production of sweat. The typical antipyretics, such as acetanilide and acetphenetidine, act after this manner increasing output. They have practically no effect against normal temperature, acting only in fever. Another drug which lowers temperature is quinine and this drug acts directly upon metabolism lessening the formation of heat.

Drugs such as strychnine or atropine which increase muscular movements, may raise the temperature, but the most important agents to increase temperature are the bacterial poisons and toxins.

The action of the antipyretics may be studied in the following experiments:—

Select three rabbits of about the same size, weighing say 1500 G. to 2000 G., and take the rectal temperature of each. The thermometer bulb should be vaselined in each case before use and should be inserted always about the same distance—say 2 inches. As the fever takes some time to develop, for afternoon use, two of the three rabbits should be injected subcutaneously at 9 A. M. with Witte's Peptone 1 G. per kilogram of body weight. The temperature should be taken each hour and plotted upon a chart. By afternoon both animals should have a well developed fever. Inject subcutaneously into one of these animals 200 mg. antipyrine and use the other fevered animal as a control. Into the third rabbit, which is normal, inject 200 mg. antipyrine and this animal will then serve as a further control in the experiment for the effect of the drug. The temperatures of the three animals should then be taken every half hour during the afternoon.

In place of using peptone to produce the fever, it is often possible to secure even more satisfactory results by the use of the increase in temperature which occurs in the anaphylactic reaction. To utilize this phenomenon, two rabbits should be sensitized by the injection of 2 cc. of beef serum two weeks before they are needed for the experiment in fever. On the day of the experiment at about 12 o'clock (for afternoon work) each of the two rabbits should be injected in the ear vein with 1 cc. of a 1-10 solution of beef serum. By 1:30 each of the rabbits will have fever. One of these fevered animals and a third (normal) rabbit are now injected with 200 mg. antipyrine and the temperature of each animal is taken every half hour and plotted on a chart as described in the earlier experiment. One of the

animals will show the normal fever curve; the second will show the curve as modified by the antipyretic, and the third curve will show the absence of effect in the case of the use of an antipyretic in an animal with a normal temperature.

Taste acetanilide, acetphenetidine and antipyrine, and test the solubility of each in water. How would they be prescribed?

Biological Assay.

One of the essential requirements in any medicine which is to be used in the treatment of disease is that not only shall it possess the properties usually found in that drug, but also that it shall be of a definite and uniform strength. With a great many drugs, such as opium and nux vomica the active principles are alkaloids and are well known, and in such cases uniform preparations are obtainable by chemical means as these alkaloids can be isolated in pure form and weighed. For instance, the Pharmacopoeia directs that 100 cc. of Tincture of Opium shall contain 1 Gram of anhydrous morphine and 100 cc. of tincture of nux vomica shall contain 0.25 grams of the alkaloids of the drug, etc. With other drugs, however, the active principles are either not known, or if known, it frequently happens that they cannot be isolated without great difficulty and in the process of isolation some of the active substance may be destroyed. To this group of drugs to which chemical standardization cannot well be applied belong the members of the digitalis group, digitalis, strophanthus, squills; ergot; cannabis indica; aconite and extracts of the suprarenal and pituitary glands.

For such drugs in place of chemical standardization methods have been introduced by which the strength of preparations is ascertained by the effects which they produce upon animal tissues. This method is known as Physiological or Biological Assay or Standardization.

On account of the importance of the subject, a brief discussion may be given of the principal methods in use. Fuller details and directions for the carrying out of the tests may be found in the Pharmacopoeia.

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For the standardization of digitalis and its allies frogs are used more frequently than any other animals. One of the most common methods and the one recommended by the Pharmacopœia is to ascertain the minimum dose of digitalis which will bring the heart of a frog to a systolic standstill in one hour. This method has been carried out in the experiment under the general subject of digitalis (Exp. VI, page 171). Fuller details are to be found in the Pharmacopœia.

Another important method of assaying digitalis is to ascertain the minimal fatal dose for a frog of a certain size, and from the knowledge of the strength of the preparation thus gained adjustments to a standard strength are made. This is the so-called "Toxic" method.

In the Focke method the time of cessation of the circulation is observed when a certain dose of digitalis is given. Again the toxic dose for guinea pigs and also for cats are obtained in still other methods of assay and adjustments made accordingly. While the two frog methods which were mentioned first are probably the most largely used of any, yet each method enumerated has certain adherents who prefer it to any other.

Ergot.

Ergot preparations are also standardized according to biological methods because on account of the complexity of its chemical constitution a standardization by chemical means is impossible. There are two principal methods in vogue. The first is to test the strength of a preparation upon an isolated uterus of a guinea pig or other animal comparing its activity with that of some known standard.

The method is described in the chapter on "Perfusion of Isolated Organs" (Page 232).

The second method for ergot depends upon the darkening or cyanosis which is produced in the cock's comb by ergot preparations.

Experiment. Select preferably Leghorn roosters weighing about 1000 G. to 1200 G. and inject into the breast muscle of one a dose of the standard preparation which is known to produce a mild degree of cyanosis of the comb. Into the breast muscle of the second rooster inject a corresponding dose of the unknown preparation and compare the intensity of the discoloration in the combs of the two birds at the end of one hour. This should give some idea as to the relative activity of the two preparations. Two or three days later inject the birds again reversing the order of the preparations upon the two birds and giving doses modified by the experience gained by the first injection so that they shall give results which are more comparable. In subsequent injections the doses are narrowed down still further until it is known exactly how much of the solution of unknown strength it is necessary to give to produce results analogous to those obtained from a certain dose of the standard. Knowing this relation it is easy to calculate how much the unknown solution must be diluted or concentrated to conform to the standard adopted. The results obtained by the isolated uterus method and by the cock's comb method run roughly parallel to each other.

Aconite.

This drug is frequently assayed biologically as its activity depends upon the presence of alkaloids differing from each other in strength. In order, therefore, to get an estimate

of the activity of the total alkaloids biological methods are necessary. The method employed is to ascertain the minimal fatal dose of the aconite preparation for a guinea pig. The standard lethal dose adopted by the Pharmacopœia for the tincture of aconite is 0.0004 cc. per gram of body weight of guinea pig. The details of the assay as described in the Pharmacopœia were carried out in the experimental work on Aconite (Page 183).

Cannabis Indica.

The assay of cannabis depends upon the fact that this drug produces in dogs certain symptoms of muscular inco-ordination. The method therefore consists of ascertaining the dose of a preparation which will produce these symptoms and then adjusting its strength to the standard requirements. The directions for carrying out the test are to be sought in the Pharmacopœia and a preparation of cannabis is to be assayed according to those directions.

Suprarenal Gland.

The assay of products of the suprarenal gland is carried out by means of a comparison of the blood pressure rise produced in dogs by an extract of the glands with that produced by a solution of known strength of the active principle of the gland. According to the standard adopted each gram of the dried suprarenals shall contain the equivalent of 10 mg. of the active principle of the gland.

The directions for carrying out this test are given in the Pharmacopœia.

Pituitary Gland.

The active principle of this gland has not been isolated in pure form and therefore products made from the gland have to be assayed biologically. The method which is used almost exclusively is the isolated uterus method which as mentioned above is used for ergot assay and which is described in the chapter on "Perfusion of isolated organs" (page 232).

The Pharmacopœia directs that 1 cc. of the solution of the Pituitary Gland shall have the same activity on the isolated uterus of a virgin guinea pig as a 1 to 20 million solution of beta-aminazoly-ethylamine hydrochloride.

A second method which is not so commonly employed is similar to that in use for suprarenal glandular products. A comparison is made of the rise in blood pressure produced by a certain dose of the product to be assayed with that produced by a standard preparation.

Pilocarpus.

Pilocarpine is an alkaloid derived from the leaves of several species of *Pilocarpus*. It has been employed in certain of the earlier experiments, which may be summarized in order to give a more complete picture of the action of the drug.

Describe the effects of pilocarpine on—

- the frog's heart (Exp. page 187);
- the turtle's heart (Exp. page 191);
- the pupil of the eye (Exp. page 247);
- the salivary secretion (Exp. page 255);
- and on the intestinal peristalsis (Exp. page 247).

How does atropine affect the activity of the drug?

I. (These drugs will be furnished you.) Take 0.003 G. of pilocarpine hydrochloride and describe any symptoms noted. Should any of these become unpleasant take atropine sulphate 0.0005 G. and describe any changes.

Other drugs which resemble pilocarpine in many of their actions are *Muscarine*, from one of the poisonous mushrooms (*Agaricus muscarius*), and *Physostigmine* or *Es-serine*, from the Calabar or Ordeal bean (*Physostigma venenosum*).

Examine the leaves of *Pilocarpus jaborandi*, and the calabar bean.

Therapeutic Uses.

The employment of pilocarpine in diseases of the eye is discussed elsewhere (page 251). The drug is practically never used for its cardiac action.

It is used to increase the activity of the sweat glands (diaphoretic action) in various diseases in which there is

an abnormal collection of fluid in the body. (Compare Diuretics, page 244). Through its action a very large amount of fluid may be excreted so that in some cases the body may lose from four to nine pounds in weight from a single dose of the drug.

Pilocarpine is employed in some diseases of the kidneys in which the urine excretion is lessened, with the idea of securing the elimination of toxic materials by the sweat glands.

The Nitrite Series.

This series comprises several drugs whose action in the body is confined almost exclusively to the blood vessels. The principal members are *Amyl Nitrite*, *Nitroglycerin*, *Sodium* and *Potassium Nitrite*.

They have been employed in various experiments and the results are to be collected and summarized.

Describe the effects of the nitrites on—

the mammalian blood pressure (Exp. page 207) ;
perfused vessels (Exp. page 227) ;
on the vessels of the intestine (Exp. page 299) ;
and on the kidney vessels (Exp. page 304).

Inhale 3 drops of amyl nitrite.

Therapeutic Uses.

The members of this series are employed in medicine to lower the blood pressure and to relieve vascular spasm.

Suprarenal Gland Extract.

The suprarenal glands contain a very active substance, *Epinephrine*, which can be isolated, but only with considerable difficulty.

For experimental work the commercial adrenalin chloride (1-1000) may be used. From one to three drops of this preparation diluted with salt solution, give good results.

Describe the effects of extract of suprarenal gland upon

the blood pressure (Exp. page 200);
the mammalian heart (Exp. page 207);
perfused vessels (Exp. page 227);
the salivary secretion (Exp. page 255);
the kidney vessels (Exp. page 304);
and upon intestinal vessels (Exp. page 299).

I. Examine the local effects of the drug by putting one drop of adrenalin chloride (1-1000) in the eye. Compare with the normal eye. How would this experiment aid you in explaining the results obtained in the experiment on blood pressure?

Therapeutic Uses.

The powerful effects of the drug on the circulation are limited in their application to therapeutics by their transitory nature and by the fact that in order to act on the heart or blood vessels (except the local action), the drug has to be injected intravenously. In some cases of shock and collapse it has been given in this way, but it requires constant administration as the effects pass off so rapidly. It is of most value for local application in surgical operations to constrict the vessels and thus render the field of operation bloodless.

Pituitary Extracts.

Extracts of the posterior lobe of the pituitary gland possess quite a powerful action which is exerted mainly upon structures containing smooth muscle. Their most important effect perhaps is upon the blood vessels where a stimulant action leads to an increased blood pressure. They also cause contractions in other organs especially in the uterus and bladder. The action is probably not upon any nerve structure but is direct upon the muscle cell.

The effect of extracts upon the circulation is studied in the experiment on page 207.

The action on smooth muscle is studied in the experiment on page 232.

Inasmuch as extracts of the gland differ considerably in strength, it is very desirable that some method of assay and of standardization should be utilized in their manufacture in order to insure a uniform strength in the glandular products. It is impossible to standardize them by chemical means at present on account of our lack of knowledge of the chemistry of the active principle of the gland. However, biological methods of assay have been adopted to a greater or less extent, and these are described in the special chapter devoted to the subject. Page 280.

The following experiments are not suitable for the regular laboratory course, but may be carried out by advanced students; they will also serve very well for class demonstrations by the instructor in charge.

Intestinal Peristalsis.¹

The peristaltic movements of the intestines and the effects of various drugs upon them may be demonstrated very well by submerging an animal in a saline solution, opening the abdomen and keeping the intestines under the surface of the water by means of a pane of glass.

For this experiment a metallic tank is needed which will be large enough to allow a rabbit to be placed in it when the animal is tied to an operating board. A tank measuring 70x30x30 cm. is large enough for most purposes and it should be mounted on legs long enough to allow a Bunsen burner to be placed under it. The operating board should be weighted with lead so that it will sink in the water. A pane of glass to cover the intestines and some small lead piping, which can be bent over the sides of the tank to act as a support for the glass, and a bath thermometer complete the special apparatus necessary for the experiment.

Fill the tank about two-thirds full of water and add sodium chloride to make a physiological salt solution (8 G. per liter). Light the Bunsen burner under the tank and heat the solution until it is of the body temperature.

¹ This experiment may have been carried out in connection with the experiment on the cervical sympathetic nerve in which case it would not be necessary to repeat it. The directions herein given, however, serve very well in case the experiment has not been carried out earlier in the course.

Operation.

In the meantime anæsthetize a rabbit or cat in the usual manner and when anæsthesia is complete insert a venous cannula in the jugular vein and also insert a tracheal cannula. Place short pieces of rubber tubing on both free ends of the tracheal cannula and close one by means of a clamp. The second piece of tubing is to extend above the surface of the water when the animal is submerged.

Place the animal in the warm salt solution which should completely cover its body. Take great care no water enters the tracheal tube, the open end of which is to be tied to a support above the tank. Make an incision through the skin and muscles in the median line of the abdomen from the end of the sternum to the symphysis. The abdominal walls may be drawn outward and secured to the operating board by means of tacks. The intestines will float to the top of the water and they must be held just beneath the surface by placing the pane of glass over them supporting the latter by means of the lead tubing.

The peristaltic movements may now be observed as well as the vascular changes which will follow the injection of the drugs named below; the changes in color of the intestines indicate the alterations in the circulation of the blood.

Inject into the jugular vein the following drugs in the order named:—

Nicotine chloride, 5 mg.,

Pilocarpine hydrochloride, 5 mg.,

Atropine sulphate, 1 mg.,

Physostigmine hydrochloride, 3 mg., injected slowly. (Observe any changes in the skeletal

muscles such as twitching. This injection may have to be repeated to secure well marked results.)

Adrenalin chloride (1-1000 sol.), 2 drops in salt solution.

Amyl nitrite, 2 or 3 drops given by inhalation.

Vasomotor Changes in the Intestine.

The special apparatus needed for this experiment is, first, some form of intestinal plethysmograph and second, an instrument to record the intestinal vascular changes upon a blackened drum.

The plethysmograph may be made very easily of plaster of Paris after the pattern devised by A. Edmunds.¹

With the plaster make a hemispherical basin ten centimeters in diameter and four deep with the walls about seven millimeters thick and with an elliptical opening in the bottom of the basin three centimeters by one and a half. Make a small opening in one side of the apparatus and cement in it a short piece of glass tubing which connects with the interior of the basin and which extends outside three or four centimeters to allow of rubber pressure tubing being connected with it. Smooth off the upper edge of the apparatus with sand paper so that a piece of glass will fit fairly tightly on it. A plethysmograph made in this way will answer all the purposes of the experiment. Its more complicated forms may be made as described in the article referred to. A glass plate which will close the top of the plethysmograph is needed as well as a piece of rubber tubing long enough to connect the plethysmograph with the recording apparatus, which may be either a tambour, a delicate piston recorder or a Brodie bellows recorder. Either of these should be provided with a side tube to allow of proper equalization of pressure on both sides of the piston or membrane of the recorder.

¹ Jour. of Physiol., Vol. XXII, 1893, page 380.

Vaseline is needed as well as a petrolate which melts at a higher temperature than vaseline and which may be made by melting the vaseline with paraffine using such proportions that the resulting mixture is fairly hard when cold.

Operation.

Anæsthetize a cat or a rabbit as usual, tie it on an operating board and insert a venous cannula.

If it is desired to take a tracing of the carotid blood pressure at the same time, the artery must be isolated, a cannula inserted and connected with the mercury manometer according to the directions given on page 200. By thus taking simultaneous tracings of the general blood pressure and of the vascular conditions in the intestinal area very instructive records may be obtained.

It is best to warm the plethysmograph slightly before applying it and to have plenty of hot salt solution ready for use as well as some absorbent cotton. Make an incision about 4 cm. long in the median line of the abdomen about midway between the sternum and symphysis and with the fingers carefully pull out of the abdominal cavity a short loop of the small intestine. Place the elliptical opening of the plethysmograph over the incision and intestinal loop and support the instrument in place with an iron ring. Draw the loop of the intestine into the apparatus until a section from 20 to 30 cm. long is lying in the basin. Cover the intestine temporarily with absorbent cotton dipped in the hot salt solution. Ligate doubly each end of the section of intestine and cut across it between the ligatures, tearing down the mesentery as far as possible and ligating any bleeding vessels. Now return to abdominal cavity the upper and lower attached ends of the

the intestine, leaving the isolated loop in the plethysmograph and connected to the animal by its section of mesentery containing the vessels and nerves.

Pack absorbent cotton rubbed up with the hardened vaseline around the mesentery so as to completely close the elliptical opening, using soft vaseline to make it air tight near the vessels; great care must be taken not to constrict the vessels. Remove the cotton covering from the intestines; vaseline the upper rim of the plethysmograph and close it with the pane of glass which has been dipped in warm salt solution to prevent the condensation of moisture on its surface.

Connect the instrument with the recorder, close the side tube on the latter and take a tracing, which, if the experiment has been properly carried out, will show both cardiac beats and the respiratory waves. Arrange the lever to write in line with the blood pressure pointer and the time marker which has been placed below.

Take tracings to show the effects of the following drugs on the general arterial blood pressure and on the vessels of the splanchnic area.

Adrenalin chloride (1-1000), 1-3 drops in salt solution.

Digitalis, tincture, 1 cc. in salt solution.

Nicotine chloride, 2 mg. in salt solution.

Amyl nitrite, 2 drops to be given by inhalation.

Pituitary extract (Pituitrin), 0.5 cc. in salt solution.

Chloroform to be given until the heart stops.

The tracing from this experiment should be studied in connection with those obtained earlier in the course in the experiment upon the dog's heart and with the results found when the vessels of the frog were perfused with salt solution.

Vascular Changes in the Kidney.

To record the changes taking place in the size of the kidney due to the differences in its blood content, an oncometer and a recording apparatus are needed. The former can be made from plaster of Paris¹ in the same manner as the intestinal plethysmograph. Several sizes should be made to accommodate the kidneys of different sized animals. A cylindrical-shaped oncometer with a flat base, having a diameter of about 5 cm. and with walls about 3.5 cm. high is large enough for the average sized cat or rabbit. One side should have a cut in it about 0.5 cm. wide and extending nearly to the bottom of the apparatus, where the cut should be widened to allow greater room for packing around the pedicle of the kidney. On the opposite side of the oncometer is a glass tube communicating with the interior of the apparatus and extending outward for the attachment of rubber pressure tubing. A piece of glass is needed to fit tightly the top of the instrument. Vaseline together with a mixture of vaseline and wax (as described on page 300) is also necessary.

For recording the volume changes on the kymograph, a tambour, piston recorder, or a bellows recorder as mentioned under the experiment on the intestinal vessels (page 299) is required. It should be fitted as before with a side tube.

¹In place of the oncometer made of plaster of Paris in the manner described a Roy kidney oncometer such as can be obtained on the market may be used, thus saving considerable time as it can be more easily adjusted than the plaster apparatus. Descriptions of the Roy oncometer may be found in works on physiology.

Operation.

Anæsthetize a rabbit or a cat and insert one cannula in the jugular vein and a second in the carotid artery. Connect the latter with the mercury manometer for blood pressure, which has been arranged according to the directions given on page 200. Either kidney may be employed in the experiment, the choice largely depending upon the arrangement of the apparatus and the light. If the left has been selected, make an incision in the abdomen well over in the left flank, extending from the costal margin downward, the length of the incision necessary depending upon the size of the oncometer. Draw the side muscles outward, if necessary continuing the cut in the muscles along the costal margin, and nail the reflected muscles to the operating board. The intestines and stomach should be packed back with absorbent cotton which has been dipped in hot salt solution and the abdominal cavity closed in as far as possible with a hot towel. This should leave the left kidney well exposed. Loosen it very carefully from all its fibrous attachments and remove the fat as far as possible, so that the kidney remains connected only by the structures entering the hilus.

Place the organ in the warmed oncometer so that the pedicle passes through the widened end of the cut in the side of the apparatus. Pack around the pedicle with absorbent cotton saturated with the hardened vaseline and completely close the opening in the side in the same way, employing the soft vaseline to make it entirely air tight. Of course it is essential to see that no undue pressure is exerted on the kidney vessels; the circulation must remain perfectly free in them. Close the top of the oncometer with the pane of glass which is made air tight with vaseline. Con-

nect the outlet tube of the instrument with rubber tubing, the other end of which is connected with the recorder. Arrange the writing point of the recorder so that it marks directly above the pointer of the blood pressure manometer and the time marker. Close the side tubes of the recorder and the lever should show the distinct cardiac pulsations as they are indicated by the changes in the kidney volume. If the pulsations do not show, either the kidney vessels are constricted or the apparatus is not air tight.

When all the apparatus is working satisfactorily inject the following drugs into the jugular vein:

Adrenalin chloride, 1-1000, 1-3 drops in salt solution.

Tincture of digitalis, 1 cc. in salt solution.

Nicotine chloride, 3 mg. in salt solution.

Amyl nitrite, 3 drops, given by inhalation.

Pituitary extract (Pituitrin), 0.5-1 cc. in salt solution.

Chloroform to be given until the heart stops.

These tracings are to be studied after the manner outlined in the section on intestinal vasomotor changes (page 303) and in connection with the tracing obtained in that experiment.

Perfusion of the Isolated Frog's Heart.

Pith the brain and the cord of a large frog weighing about 75 grams. Tie it on the frog board and expose the heart in the usual manner. Pass a fine ligature around both aortae. With a fine pair of scissors make a V-shaped incision in one of the aortae, insert a cannula pointing towards the heart and tie it in, tying both aortae at the same time. With a fine glass capillary pipette wash the blood out of the cannula with Ringer solution until it is clear. Divide the aortae. Divide the connections of the heart with the posterior wall of the thorax avoiding cutting any of the vessels. Pass a ligature around all the remaining vessels and turn the heart up so as to expose the ascending vena cava into view. Tie a cannula into it as low down as possible, pointing towards the heart, and tying off all the other vessels at the same time. Fill the cannula with Ringer solution, and remove the heart from the body.

The perfusion apparatus consists of a double reservoir provided with Mariotte stoppers, rubber tubing, and a 3-way stop-cock. Place 100 cc. of Ringer solution in one reservoir, and the same amount of Ringer solution to which 2 to 3 drops of 1-1000 adrenaline solution have been added, in the other. Connect the venous cannula with the perfusion apparatus making sure that all air bubbles have been excluded. The cannula inserted into the aorta is to be held in position by a clamp. The tip of the ventricle is attached by means of a fine spring clasp and silk thread to a light recording lever. The pressure of the perfusion fluid should not exceed 6 cm., and the flow of the perfusion fluid into the

heart is adjusted by means of a screw clamp, and is to be maintained constant throughout the experiment. A rate of flow so that 1-2 drops are expelled with each systole will probably be most satisfactory. The resistance against which the heart is to contract is adjusted by properly tipping the cutflow cannula, and this should likewise be kept constant.

After obtaining a normal record of the heart action turn on the adrenalin perfusion fluid and note the effects of this drug upon the heart. When this has been noted turn on Ringer solution until the heart has returned to its normal condition. Now add to the fresh Ringer solution 0.5 cc. of a 1-1000 solution of aconitine hydrochloride. Continue the perfusion with this drug until the heart stops.

Other drugs that may be tried are:

Cocaine hydrochloride, 1-25000.

Calcium chloride, 1-500.

Digitalein, 1-20000.

Ammonium chloride, 1-1000.

until such time as you are ready to proceed with the experiment.

The defibrinated blood is strained through cotton and diluted with warmed (38°C.) Ringer-Locke solution to make a total volume of about 200 cc. (From 10 to 15% blood will give best results.) Pass a stream of oxygen through the diluted blood for a few minutes. To 100 cc. of the oxygenated blood solution add 0.2 cc. of 1-1000 solution of ouabain and transfer this to one reservoir. Into the other reservoir place 100 cc. of normal oxygenated blood. Fill the whole system with the perfusion fluids, excluding rigorously all air bubbles. Tie the cannula of the perfusion apparatus into the aorta, making sure that it does not penetrate the semilunar valves. Connect the apex of the heart with a recording lever by means of a hook and silk thread, and start the perfusion of the coronaries with the normal blood solution at a constant temperature of 38° C. The perfusion fluid is to be caught in a large beaker and is to be returned to the reservoir as often as may be necessary. At the beginning of the experiment the perfusion fluid will run through rather rapidly, and the heart will probably beat quite irregularly. After the heart has become perfectly regular, and a record has been taken on the revolving drum, 2 to 3 drops of a 1-10000 solution of adrenalin may be added to the normal perfusion fluid. Note its effects upon the heart, especially with regard to the amplitude, rate of beat, and the rate of the outflow of the perfusion fluid.

After the effects of adrenalin have been studied, turn on the ouabain perfusion fluid, and study the various stages of digitalis action upon the heart. Continue the perfusion with this drug until the heart has stopped beating and for 5 to 10 minutes thereafter.

DRUGS AND REAGENTS REQUIRED FOR EACH EXPERIMENT.

Anæsthetics: Page 11.

Ether.

Chloroform.

Paraldehyde.

Chloretone in alcohol or olive oil.

Urethane.

Morphine Sulphate.

Anticoagulating Solutions: Page 17.

Sodium Sulphate, half saturated.

Sodium citrate. 5-10%.

Magnesium Sulphate, 25%.

General Reagents Needed:

Alcohol.

Chloroform.

Ether.

Dilute Sulphuric Acid.

Potassium Carbonate, 10%.

Potassium Hydroxide, 10%.

Alkaloidal Reagents.

Tannic Acid, 10%.

Picric Acid, Saturated.

Iodine in Potassium Iodide, U. S. P.

Mercury Potassium Iodide, U. S. P.

Phosphotungstic Acid, 2%.

Fehling's Solution, U. S. P.

Ferric Chloride Solution.

Concentrated Acids.

Alkaloids: Page 20.

Quinine.

Quinine Sulphate.

Glucosides: Page 28.

Amygdalin.

Bitter Almonds.

Volatile Oils: Page 32.

Oil of Turpentine.

Cotton Seed Oil.

Resins: Page 39.

Powdered resin.

- Saponins: Page 40.
 - Saponin, 2%.
 - Castor Oil.
- Powders: Page 51.
 - Calomel.
 - Sugar of Milk.
- Capsules: Page 55.
 - Quinine Sulphate.
 - Oil of Turpentine.
- Cachets: Page 59.
 - Quinine Sulphate.
- Pills: Page 60.
 - Extract of Gentian.
 - Starch.
 - Lycopodium.
- Emulsions: Page 67.
 - Bitter Almonds.
 - Asafoetida.
 - Castor Oil.
 - Powdered Acacia.
- Ointments: Page 72.
 - Extract of Belladonna.
 - Benzoinated lard.
 - Hydrous wool fat.
- Suppositories: Page 76.
 - Oil of Theobroma.
 - Tannic Acid.
 - Lycopodium.
- Physiology of Frog: Page 80.
 - Acid fuchsin, 1.5%.
- Soporifics: Page 88.
 - Chloral.
 - Sulphonal.
 - Paraldehyde.
 - Veronal.
- Nux Vomica: Page 96.
 - Powdered nux vomica.
 - Strychnine Sulphate.
 - Strychnine Sulphate, 1/10% Solution.
 - Potassium Bichromate.
 - Manganese Dioxide.
- Camphor Group; Page 112.
 - Camphor.
 - Thujon.
 - Picrotoxin.

- Opium: Page 116.
 - Morphine Sulphate.
 - Cane Sugar.
 - Iodic Acid, 5% solution.
 - Starch Paste.
 - Codeine phosphate, 1.5% solution.
- Curara: Page 127.
 - Curara Solution.
- Nicotine: Page 132.
 - Nicotine Chloride Solution.
- Veratrine: Page 135.
 - Veratrine Sulphate, 0.1% solution.
- Caffeine Group; Page 136.
 - Caffeine.
 - Sodium Benzoate.
 - Theobromine-Sodium Salicylate.
- Cocaine: Page 143.
 - Cocaine Hydrochloride.
 - Cocaine Hydrochloride, 4% solution.
- Belladonna Group: Page 147.
 - Powdered Belladonna Leaves.
 - Atropine Sulphate.
 - Potassium Hydroxide in Alcohol.
- Cinchona: Page 156.
 - Quinine.
 - Quinine Sulphate.
 - Quinine Hydrochloride.
 - Chlorine Water.
- Anæsthetics: Page 160.
 - Nitrous Oxide.
 - Ethyl Chloride.
 - Magnesium Sulphate.
 - Sodium Oxalate.
 - Calcium Chloride, 3% solution.
- Digitalis Group: Page 168.
 - Powdered Digitalis leaves.
 - Bruised Digitalis leaves.
- Aconite: Page 183.
 - Tincture of Aconite.
- Drugs on Frog's Heart: Page 187.
 - Pilocarpine Nitrate, 0.5% solution.
 - Atropine Sulphate, 0.1% solution.

- Drugs on Turtle's Heart: Page 191.
Nicotine Solution.
Pilocarpine Solution.
Atropine Solution.
- Drugs on Blood Pressure: Page 200.
Amyl Nitrite.
Suprarenal Gland Extract.
Nicotine Chloride Solution.
Pituitary Gland Extract.
Tincture of Digitalis.
Barium Chloride, 2% solution.
- Drugs on Mammalian Heart: Page 207.
Same as for "Blood Pressure."
- Digitalis in Auricular Fibrillation: Page 220.
Tincture of Digitalis.
- Perfusion of Blood Vessels: Page 227.
Sodium Nitrite.
Suprarenal Gland Extract.
- Drugs on Isolated Tissues: Page 232.
Suprarenal Gland Extract.
Pilocarpine Solution.
Atropine Solution.
Fluid Extract of Ergot.
Pituitary Gland Extract.
- Diuresis: Page 239.
Theocin.
Sodium Iodide.
Sodium Nitrite, 10% solution.
Fehling's Solution.
Sodium Nitrate, 5% solution.
- Cervical Sympathetic System: Page 247.
Nicotine Chloride Solution.
Pilocarpine, 0.5% solution.
Atropine, 0.1% solution.
Suprarenal Gland Extract.
- Salivary and Pancreatic Secretion: Page 255.
Same as for Cervical Sympathetic System.
Secretin.
- Antipyretics: Page 267.
Peptone.
Beef Serum.
Antipyrine.
Acetanilide.
Acetphenetidine.

Biological Assay: Page 272.

Fluid Extract Cannabis.
Fluid Extract Ergot.
Suprarenal Gland Extract.
Pituitary Gland Extract.

Intestinal Peristalsis: Page 292.

Same as for Cervical Sympathetic System.
Physostigmine Hydrochloride.
Amyl Nitrite.

Vasomotor Changes in the Intestine: Page 299.

Suprarenal Gland Extract.
Tincture of Digitalis.
Nicotine Chloride Solution.
Amyl Nitrite.
Pituitary Gland Extract.

Vasomotor Changes in the Kidney: Page 304.

Same as for the Intestine.

Isolated Frog's Heart: Page 311.

Cocaine Hydrochloride.
Calcium Hydrochloride.
Digitalein.
Ammonium Chloride.

Isolated Mammalian Heart: Page 315.

Suprarenal Extract.
Ouabain.

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